FORMULATION AND *IN-VITRO* EVALUATION OF 5-FLUOROURACIL MICROCAPSULES BY USING DIFFERENT METHODS OF MICROENCAPSULATION

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MASTER OF PHARMACY (Pharmaceutics)

Submitted by

SRIKANTH REDDY JEDDIPELLY

(Register No: 26116012)

Under the Guidance of

Dr. S.SHANMUGAM, M. Pharm., Ph.D.

Professor, Department of Pharmaceutics

ADHIPARASAKTHI COLLEGE OF PHARMACY

(ACCREDITED BY "NACC" WITH A CGPA OF 2.74 ON A FOUR POINT SCALE AT "B" GRADE)

MELMARUVATHUR - 603 319

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CERTIFICATE

This is to certify that the research work entitled "**FORMULATION AND *INITRO* EVALUATION OF 5-FLUOROURACIL MICROCAPSULES BY USING DIFFERENT METHODS OF MICROENCAPSULATION"** submitted to The Tamil Nadu Dr.M.G.R. Medical University, Chennai in partial fulfillment for the award of the Degree of the Master of Pharmacy (Pharmaceutics) was carried out by **"SRIKANTH REDDY JEEDIPELLY" (Register No. 26116012)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2012-2013.

Place:Melmaruvathur
Date:

Prof. (Dr.) S.SHANMUGAM, M. Pharm., Ph.D.
Department of Pharmaceutics,
Adhiparasakthi College of Pharmacy,
Melmaruvathur - 603 319.

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND *IN-VITRO* EVALUATION OF 5-FLUOROURACIL MICROCAPSULES BY USING DIFFERENT METHODS OF MICROENCAPSULATION"** the Bonfide research work carried out by **"SRIKANTH REDDY JEDDIPELLY" (Register No. 26116012)** in the Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, Melmaruvathur which is affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, under the guidance of **Dr.S.SHANMUGAM,** M.Pharm.,Ph.,D. Department of Pharmaceutics, Adhiparasakthi College of Pharmacy, during the academic year 2012-2013

Place: Melmaruvathur
Date:

Prof. (Dr.) T. VETRICHELVAN, M. Pharm., Ph.D.,
Principal,
Adhiparasakthi College of Pharmacy,
Melmaruvathur - 603 319.

Dedicated

To

All cancer patients...

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SRIKANTH REDDY JEDDIPELLY

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ABBREVIATIONS

%	----	Percentage
<	----	Less Than
>	----	More Than
°C	----	Degree Celsius
µg	----	Microgram
cm	----	Centimeter
DE	----	Dissolution Efficiency
DSC	----	Differential Scanning Calorimetry
F	----	Formulation
FTIR	----	Fourier Transform-InfraRedSpectroscopy
GIT	----	Gastrointestinal Tract
gm	----	Grams
HCl	----	Hydrochloric acid
HPMC	----	Hydroxypropyl methylcellulose
hrs	----	Hours
ICH	----	International Conference on Harmonization
IP	----	Indian Pharmacopoeia
MDT	----	Mean Dissolution Time
mg	----	Milligram
ml	----	Milliliter
mm	----	Millimeter
N	----	Normality

nm	----	Nanometer
NSAID	----	Non-Steroidal Anti-Inflammatory Drugs
PBS	----	Phosphate Buffer Solution
RH	----	Relative Humidity
rpm	----	Revolutions per Minute
S. No.	----	Serial Number
SEM	----	Scanning electron microscope
T	----	Time
USP	----	United State Pharmacopoeia
UV	----	Ultra Violet
W/v	----	weight/volume
λmax	----	Absorption maximum

INTRODUCTION...

1. INTRODUCTION

(Khachane K.N. et al.. 2011, Shalin A. Modi, et al..2011)

Oral route has been one of the most popular routes of drug delivery dueto its easeof administration, patience compliance and least sterility constraints and flexible design of dosage forms. Time release technology, also known as sustained-release (SR), sustained-action (SA), extended-release(ER), time-release ortimed-release, controlled-release(CR), modifiedrelease (MR) or continuous-release (CR), is a mechanism used in pill tablets or capsules to dissolve slowly and release a drug overaprolong period oftime. Different polymers are employed dueto their insitugel forming characteristics and their ability to release entrapped drug in the specific medium by swelling and cross-linking. Hydrophilic polymer matrix is widely used for formulating an SRdosageform. Because of increased complication and expense involved in marketing of newdrug entities, has focused greater attention on development of sustained release or controlled releasedrug delivery system. Matrix system is widely used for the purpose of sustainedrelease. Infact, a matrix is defined as a well-mixed composite of one or more drugs with gelling agent i.e. hydrophilic polymers. By the sustained release method therapeutically effective concentration can be achieved in the systemic circulation over an extended period of time, thus achieving better compliance of patients. Sustained release dosage forms are prepared by coating the tablets so that the rate of solubility is controlled or individual encapsulating microparticles of varying size sothat the rate of dissolution can be controlled. With the development of modern synthetic ion exchange resins, pharmaceutical industry adapted the ion exchange technology to achieve sustained release of drug.

1.1 Concept of Sustained Release (SR): (*Kranthi Kumar Kotta.et al..2010)*

The object of sustain release of drugs, in a general way is to modify the normal behavior of the drug molecule in physiological environment. The following are the benefits of sustained release formulations.

1. Sustained action at predetermined rate by maintaining a relatively constant, effective drug level in the body with minimum side effects
2. Localization of drug action by special placement of a controlled release systems usually rate controlled adjacent to or in diseased tissue of organ.
3. Targeting drug action by using or chemical derivatives to deliver drug to particular target cell type.

1.1.1 sustained release drug delivery system: (Remington., 2002)

Non immediate release drug delivery system may be conveniently divided into four categories.

i. Delayed release
ii. Sustained release
    a. Controlled release
    b. Prolonged release
iii. Site specific release
iv. Receptor release

Sustained release system is a drug delivery that achieves release of drug over an extended period of time. If the system is successful at maintaining controlled drug level in the blood, it is considered as a controlled release system. If it is unsuccessful but extends the duration of action over that achieved by conventional delivery it is considered as a prolonged release system.

1.1.2 Advantages of Sustained Release Formulations (Sharma Nk., 1998)

1. Overcome patient compliance problems.

2. Minimize or eliminate systemic side effects by reduced fluctuation in drug level.

3. Minimize drug accumulation with chronic dosing.

4. Improve efficiency in treatment

   a) Cures or controls disease condition more promptly.

   b) Improves therapy and reduce the undesirable side effect by maintains the drug level in plasma for prolonged period of time.

   c) Improves bioavailability of some drugs.

5. Economy i.e. reduction in health care costs. The average cost of treatment over an extended time period may be less.

6. Reduce dose frequency

7. Reduce fluctuations in blood levels

1.1.3 Disadvantages of Sustained Release Formulations:

1) Decreased systemic availability in comparison to immediate release conventional dosage forms, which may be due to incomplete release, increased first-pass metabolism, increased instability, insufficient residence time for complete release, site specific absorption, pH dependent stability etc.

2) Poor in vitro – in vivo correlation.

3) Retrieval of drug is difficult in case of toxicity, poisoning or hypersensitivity reactions.

4) Reduced potential for dose adjustment of drugs normally administered in varying strengths.

1.1.4 Classification of sustained release delivery system:

1. Rate program drug development systems

2. Activated modulated drug development systems.

3. Feed base modulated drug development systems.

4. Site targeting drug development

All categories consist of common structural features.

i. Drug reservoir compartment

ii. Rate controlling element

iii. Energy source

1.1.5 Attributes of drug candidates for sustained release systems:

There are specific attributes that a drug must possess for being suitable for incorporation in sustained release systems.

1. The drug must be effective in a relatively small dose or else the large dose required will make the preparation difficult to swallow.
2. Drugs with very short biological half life (less than 2 hrs) such as levodopa, penicillin G, and furosemide require relatively large dose for incorporating in sustain Release systems. His renders the dosage form very difficult to swallow.
3. Drugs with long biological half live (more than8 hrs) inherently or sustain release and thus are viewed as questionable candidates for sustained release formulations.
4. Absorption of poorly water soluble drugs is often limited by dissolution rate. Incorporation of such drugs into sustained release formulations is therefore unnecessary and is likely to reduce the overall absorption efficiency.
5. Very insoluble drugs whose availability is controlled by dissolution (example griseofulvin) may not benefit from this, since the amount of drug available for absorption is limited by the poorly solubility of the compound.

6. Drugs with narrow requirement for absorption (e.g. drugs dependent on position in the GI tract for optimum absorption) are also poor candidates for oral sustained release formulations, since absorption must occur throughout the length of the gut. E.g. vitamin C is absorbed preferentially from the upper portion of the intestine and therefore it's sustain release formulation are of questionable therapeutic value.
7. Before proceeding with the design of sustained release form of an appropriate drug, the formulated should have an understanding the pharmacokinetics of the candidate, should be that pharmacologic effect can be positively correlated with drug blood levels, and should be knowledgeable about the therapeutic dosage, including the minimum effective and maximum safe doses.

Although the above characteristic are useful rules of thumb for deciding whether or not particular drug should be considered for sustained release drug delivery system, there are several exceptions biological half life of nitroglycerin is less than 0.5hrs. it is rapidly metabolized in liver and is poorly absorbed orally. However, sustain release oral nitroglycerin obtained from these products provide adequate prophylaxis against anginal attacks but are inadequate to treat acute anginal episodes.

1.2 Factors Influencing the Design and Performance of Sustained Release Products: *(Bramhankar and Jaiswal, 1995)*

The type of delivery system and route of administration of the drug presented in sustained drug delivery system may depend upon two properties They are

I. Physicochemical Properties of drugs
II. Biological Factors.

1.2.1 Physicochemical Properties of Drugs (*Shalin A. Modi, et al..2011)*

1. Dose size:

If an oral product has a dose size greater that 0.5gm it is a poor candidate for sustained release system, Since addition of sustaining dose and possibly the sustaining mechanism will, in most cases generates a substantial volume product that unacceptably large.

2. Ionization, P^{Ka} and Aqueous Solubility:

The pH Partition hypothesis simply states that the unchanged form of a drug species will be preferentially absorbed through many body tissues. Therefore it is important to note the relationship between theP^{Ka} of the compound and its absorptive environment. For many compounds, the site of maximum absorption will also be the area in which the drug is least soluble.

3. Partition coefficient:

The compounds with a relatively high partition coefficient are predominantly lipid soluble and easily penetrate membranes resulting high bioavailability. Compounds with very low partition coefficient will have difficulty in penetrating membranes resulting poor bioavailability. Furthermore partitioning effects apply equally to diffusion through polymer membranes.

4.Drug Stability: *(Asija Rajesh, et al.. 2012, Shalin A. Modi, et al..2011)*

In general the drugs, which are unstable in GIT environment poor candidates for oral sustained release forms. Orally administered drugs can be subject to both acid base hydrolysis and enzymatic degradation. Degradation will proceed at the reduced rate for drugs in the solid state, for drugs that are unstable in stomach; systems that prolong delivery ever the entire course of transit in GI tract are beneficial.

Compounds that are unstable in the small intestine may demonstrate decreased bioavailability when administered form a sustaining dosage from. This is because more drug is delivered in small intestine and hence subject to degradation.

5. Protein Binding:

It is well known that many drugs bind to plasma proteins with a concomitant influence on the duration of drug action. Since blood proteins are mostly recalculated and not eliminated. Drug protein binding can serve as depot for drug producing a prolonged release profile, especially if a high degree of drug binding occurs. Extensive binding to plasma proteins will be evidenced by a long half life of elimination for drugs and such drugs generally do not require a sustained release dosage form.

6. Molecular size and diffusivity:

The ability of drug to diffuse through membranes it's so called diffusivity & diffusion coefficient is function of molecular size (or molecular weight).Generally, values of diffusion coefficient for intermediate molecular weight drugs, through flexible polymer range from 10-8 to 10-9 cm^2/sec. with values on the order of 10-8 being most common for drugs with molecular weight greater than 500, the diffusion coefficient in many polymers frequently are so small that they are difficult to quantify i.e. less than 16-12 cm^2/sec. Thus high molecular weight drugs and/or polymeric drugs should be expected to display very slow release kinetics in sustained release device using diffusion through polymer membrane.

1.2.2 II. Biological Factors **(***Shalin A. Modi, et al..2011)*

1. Biological Half-Life:

Therapeutic compounds with half-life less than 8 hrs are excellent candidates for sustained release preparations. Drugs with very short half-life (less than 2 hrs) will require excessively large amounts of drug in each dosage unit to maintain controlled effects. Compounds with relatively long half-lives, generally greater than 8 hrs are not used in the sustained release dosage forms, since their effect is already sustained and also GI transit time is 8-12 hrs (Jantzen*et al.*. 1996). So the drugs, which have long -half life and short half- life, are poor candidates for sustained release dosage forms.

4. Absorption:

The characteristics of absorption of a drug can greatly affect its suitability as a sustained release product. Drugs which are absorbed by specialized transport process (carrier mediated) and drug absorption at special sites of the gastrointestinal tract (Absorption Window) are poor candidates for sustained release products.

5. Distribution:

The distribution of drugs into tissues can be important factor in the overall drug elimination kinetics. Since it not only lowers the concentration of circulating drug but it also can be rate limiting in its equilibrium with blood and extra vascular tissue, consequently apparent volume of distribution assumes different values depending on time course of drug disposition. For design of sustained/controlled release products, one must have information of disposition of drug.

6. Metabolism:

There are two factors associated with the metabolism of some drugs; however that present problems of their use in sustained-release systems. One is the ability of the drug to induce or inhibit enzyme synthesis; this may result in a fluctuating drug blood level with chronic dosing. The other is a fluctuating drug blood level due to intestinal (or other tissue) metabolism or through a hepatic first-pass effect.

Drugs that are significantly metabolized especially in the region of the small intestine can show decreased bioavailability from slower releasing dosage forms. The drugs should not have intestinal first pass effect and should not induce (or) inhibit metabolism are good candidates for sustained release dosage forms.

1.3 Sustained (zero-order) drug release has been attempted to be achieved with various classes of sustained drug delivery system (*Caugh Isha, et al.. 2012*)

1. Diffusion sustained system.
    i) Reservoir type.
    ii) Matrix type
2. Dissolution sustained system.
    i) Reservoir type.
    ii) Matrix type
3. Methods using Ion-exchange.
4. Methods using osmotic pressure.
5. pH independent formulations.
6. Altered density formulations.

1.3.1. Diffusion Sustained System (*Brahmankar., 2005*)

Basically diffusion process shows the movement of drug molecules from a region of a higher concentration to one of lower concentration. The flux of the drug J (in amount / area - time), across a membrane in the direction of decreasing concentration is given by Fick's law.

$J = - D \, dc/dx$.

D = diffusion coefficient in area/ time

dc/dx = change of concentration 'c' with distance 'x'

In common form, when a water insoluble membrane encloses a core of drug, it must diffuse through the membrane.

The drug release rate dm/ dt is given by

$dm/dt = ADK\Delta C/L$

Where,

A = Area.

K = Partition coefficient of drug between the membrane and drug core.

L = Diffusion path length (i.e. thickness of coat).

ΔC = Concentration difference across the membrane.

i) Reservoir Type (Khachane K.N, et al.. 2011)

In the system, a water insoluble polymeric material encases a core of drug (Figure 1.1). Drug will partition into the membrane and exchange with the fluid surrounding the particle or tablet. Additional drug will enter the polymer, diffuse to the periphery and exchange with the surrounding media.

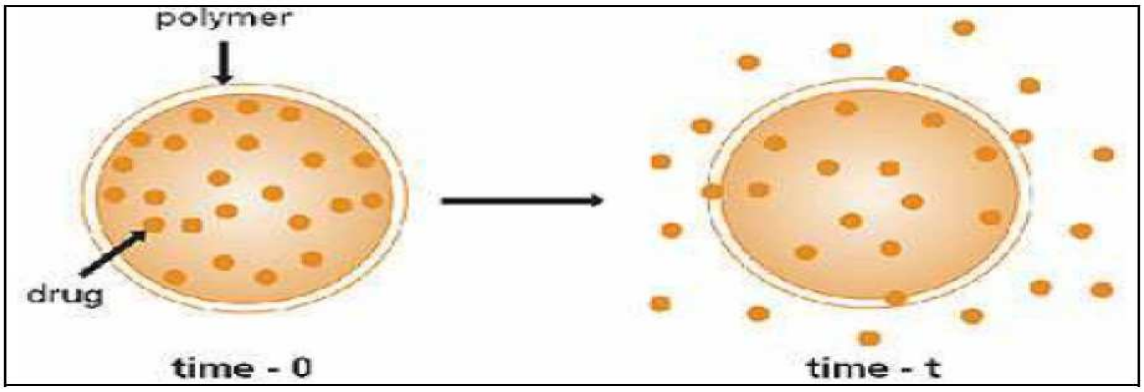


Fig 1.1: Schematic representation of diffusion sustained drug release: Reservoir system

ii) Matrix Type **(***Caugh Isha, et al.. 2012*)

A solid drug is dispersed in an insoluble matrix and the rate of release of drug is dependent on the rate of drug diffusion and not on the rate of solid dissolution. Higuchi has derived the appropriate equation for drug release for this system:

$Q = D\varepsilon / T [2 A - \varepsilon Cs] Cst^{1/2}$

Where;

Q = Weight in gms of drug released per unit area of surface at time t.

D = Diffusion coefficient of drug in the release medium.

ε = Porosity of the matrix.

Cs = Solubility of drug in release medium.

T = Tortuosity of the matrix.

A = Concentration of drug in the tablet, as gm/ ml.

The release rate can be given by following equation

Release rate = AD / L = [C1- C2]

Where;

A = Area.

D = Diffusion coefficient.

C1 = Drug concentration in the core.

C2 = Drug concentration in the surrounding medium.

L = Diffusional path length.

Thus diffusion sustained products are based on two approaches the first approach entails placement of the drug in an insoluble matrix of some sort. The eluting medium penetrates the matrix and drug diffuses out of the matrix to the surrounding pool for ultimate absorption. The second approach involves enclosing the drug particle with a polymer coat. In this case the portion of the drug which has dissolved in the polymer coat diffuses through an unstirred film of liquid into the surrounding fluid.

1.3.2 Dissolution Sustained Systems **(***Caugh Isha, et al.. 2012*)

A drug with a slow dissolution rate is inherently sustained and for those drugs with high water solubility, one can decrease dissolution through appropriate salt or derivative formation. These systems are most commonly employed in stomach from the effects of drugs such as Aspirin; a coating that dissolves in natural or alkaline media is used. This inhibits release of drug from the device until it reaches the higher pH of the intestine. In most cases, enteric coated dosage forms are not truly sustaining in nature, but serve as a useful function in directing release of the drug to a special site. The same approach can be employed for compounds that are degraded by the harsh conditions found in the gastric region.

i) Reservoir Type

Drug is coated with a given thickness coating, which is slowly dissolved in the contents of gastrointestinal tract. If the outer layer is quickly releasing bolus dose of the drug, initial levels of the drug in the body can be quickly established with pulsed

intervals. Although this is not a true sustained release system, the biological effects can be similar. An alternative method is to administer the drug as group of beads that have coating of different thickness. Since the beads have different coating thickness, their release occurs in a progressive manner. Those with the thinnest layers will provide the initial dose. The maintenance of drug levels at late times will be achieved from those with thicker coating. This is the principle of the spansule capsule. Cellulose nitrate phthalate was synthesized and used as an enteric coating agent for acetyl salicylic acid tablets.

ii) Matrix Type

The more common type of dissolution sustained dosage form as shown in fig 1.2. It can be either a drug impregnated sphere or a drug impregnated tablet, which will be subjected to slow erosion.

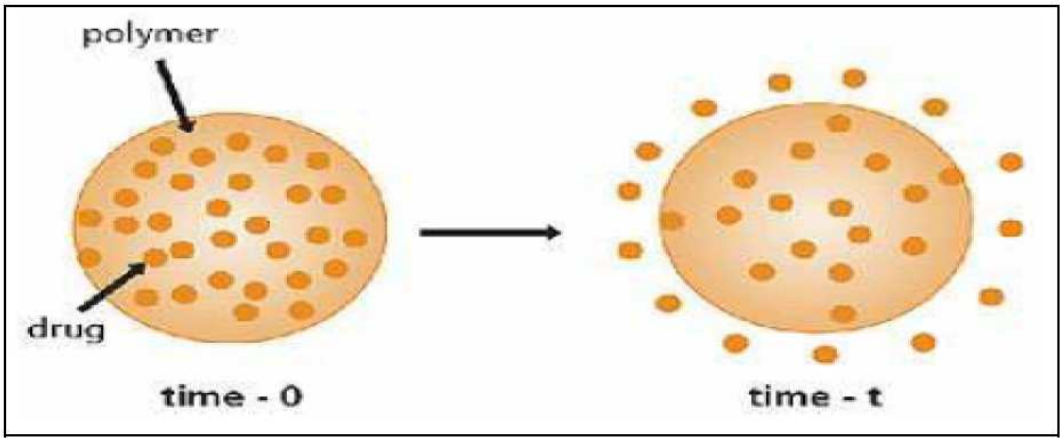


Fig 1.2: Schematic representation of diffusion sustained drug release: matrix system

Two types of dissolution sustained pulsed delivery systems

(*Caugh Isha, et al.. 2012*)

- Single bead type device with alternating drug and rate controlling layer.
- Beads containing drug with differing thickness of dissolving coats. Amongst sustained release formulations, hydrophilic matrix technology is the most widely used drug delivery system due to following advantages:

- Provide desired release profiles for a wide therapeutic drug category, dose and solubility.
- Simple and cost effective manufacturing using existing tableting unit operation equipment.
- Robust formulation.
- Broad regulatory and patient acceptance.
- Ease of drug release modulation through level and choice of polymeric systems and function coatings.

1.3.3. Methods using Ion Exchange

It is based on the formation of drug resin complex formed when a ionic solution is kept in contact with ionic resins. The drug from these complexes gets exchanged in gastrointestinal tract and released with excess of Na+ and Cl- present in gastrointestinal tract.

Anion Exchangers: Resin+ - Drug - + Cl- goes to Resin+ Cl- + Drug-

Cation Exchangers: Resin- - Drug+ + Na+ goes to Resin- Na+ + Drug+

These systems generally utilize resin compounds of water insoluble cross linked polymer. They contain salt forming functional group in repeating positions on the polymer chain. The release rate can be sustained by coating the drug resin complex by microencapsulation process.

1.3.4. Methods Using Osmotic Pressure **(***Caugh Isha, et al.. 2012*)

A semi permeable membrane is placed around a tablet, particle or drug solution that allows transport of water into the tablet with eventual pumping of drug solution out of the tablet through a small delivery aperture in tablet coating.

Two types of osmotically sustained systems are

- Type A contains an osmotic core with drug.

- Type B contains the drug in flexible bag with osmotic core surrounding.

1.3.5. pH– Independent Formulations

The gastrointestinal tract present some unusual features for the oral route of drug administration with relatively brief transit time through the gastrointestinal tract, which constraint the length of prolongation, further the chemical environment throughout the length of gastrointestinal tract is constraint on dosage form design. Since most drugs are either weak acids or weak bases, the release from sustained release formulations is pH dependent. However, buffers such as salts of amino acids, citric acid, phthalic acid phosphoric acid or tartaric acid can be added to the formulation, to help to maintain a constant pH thereby rendering pH independent drug release. A buffered sustained release formulation is prepared by mixing a basic or acidic drug with one or more buffering agent, granulating with appropriate pharmaceutical excipients and coating with gastrointestinal fluid permeable film forming polymer. When gastrointestinal fluid permeates through the membrane, the buffering agents adjust the fluid inside to suitable constant pH thereby rendering a constant rate of drug release e.g. propoxyphene in a buffered sustained release formulation, which significantly increase reproducibility.

1.3.6. Altered Density Formulations **(***Caugh Isha, et al.. 2012***)**

It is reasonable to expect that unless a delivery system remains in the vicinity of the absorption site until most; if not all of it would have limited utility. To this end, several approaches have been developed to prolong the residence time of drug delivery system in the gastrointestinal tract.

High Density Approach

In this approach the density of the capsules must exceed that of normal stomach content and should therefore be at least 1-4gm/cm3.

Low Density Approach

Globular shells which have an apparent density lower than that of gastric fluid can be used as a carrier of drug for sustained release purpose.

1.4Rationale for the selection of Microparticles:

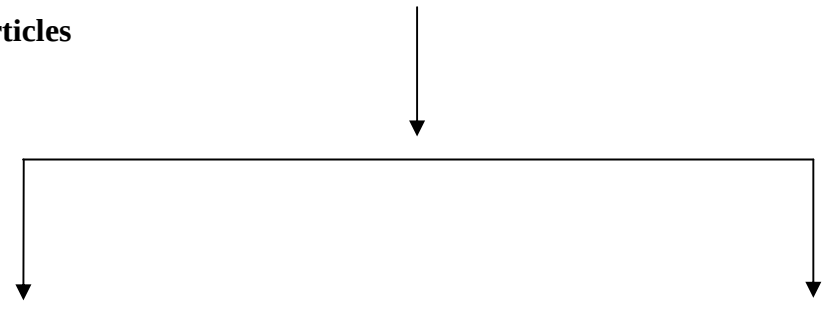
Most of the research effort in developing novel drug delivery systems has been focused on oral controlled release dosage forms. Among them, in the last decade, multiple unit dosage forms, such as beads or micro particles. Have gained in popularity for different reasons when compared to non-disintegrating single-unit dosage forms. They distribute more uniformly in the gastrointestinal tract, resulting in more uniform and reduce local irritation, and also avoid the unwanted intestinal retention.

1.4.1 Micro particles:

These are particles with size more than '1' µm, containing the polymer. At present, there is no universally accepted size range that particles must have in order to be classified as micro particles. However, may workers classify the particles smaller than '1' µm, as nanoparticles as and those more than 1000 µm, as macro particles.

Classification: Micro particles are classified into two groups.

Micro particles



Microcapsules **Microspheres**

(Micrometric Reservoir System) (Micrometric Matrix System)

1.4.2 Microcapsules: (Nitika Agnihotri, et al..2012)

Microcapsules are small particles that contain an active agent or core material surrounded by a coating or shell. (Commercial microcapsules typically have a diameter between 3 & 800 micrometer and 10-90% core).

1.4.3 Microspheres:

Microspheres are solid, spherical particles containing dispersed drug molecules, either in solution or crystalline form, among the polymer molecule.

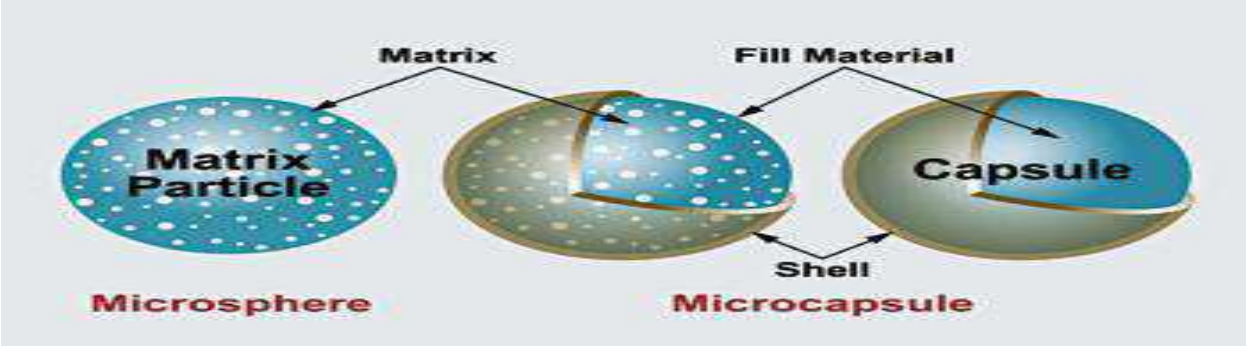
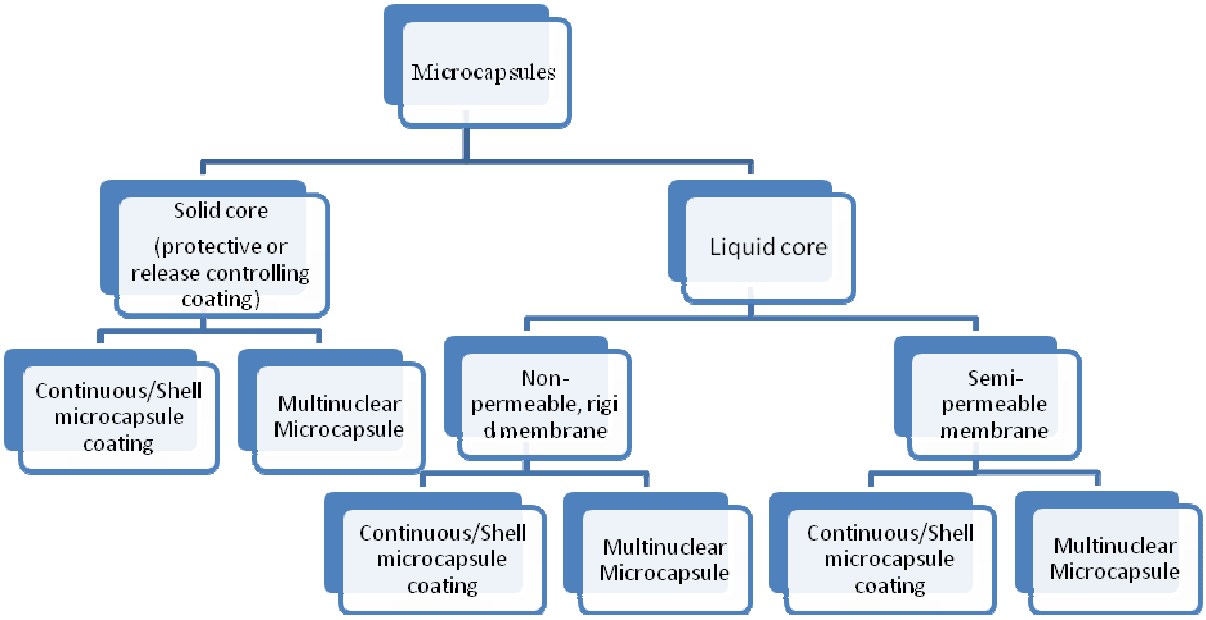


Fig 1.3: Microsphere & microcapsule

1.4.4 TYPES OF MICROCAPSULES:



Microcapsules have an either spherical geometry with a continuous core region surrounded by a continuous shell or have an irregular geometry and contain a number of small droplets or particles of core.

Reasons for Encapsulation:

There are several reasons why substances may be encapsulated

1. To protect reactive substances from the environment

2. To convert liquid active components into a dry solid system

3. To separate incompatible components for functional reasons

4. To mask undesired properties of the active components

5. To protect the immediate environment of the microcapsules from the active components

6. To control release of the active components for delayed (timed) release or long-acting (sustained) release

1.5 CRITERIA FOR COATING MATERIALS:

The coating materials should meet the following ideal criteria:-

1. Capable of forming a film that is cohesive with the core material.
2. Chemically compatible and non-reactive with the core material.
3. Provide the desired coating properties such as strength, flexibility, impermiability, optical properties and stability.

The selection of a given coating material often can be aided by the review of existing literature and by the study of free or cast films.

1.6 Release mechanisms . (*Christopher S. Brazel, et al.. 2010)*

Mechanisms of drug release from microcapsules are

1. **Degradation controlled monolithic system:**

The drug is dissolved in matrix and is distributed uniformly throughout. The drug is strongly attached to the matrix and is released on degradation of the matrix. The diffusion of the drug is slow as compared with degradation of the matrix.

2. Diffusion controlled monolithic system

Here the active agent is released by diffusion prior to or concurrent with the degradation of the polymer matrix. Rate of release also depend upon where the polymer degrades by homogeneous or heterogeneous mechanism.

3. Diffusion controlled reservoir system

Here the active agent is encapsulated by a rate controlling membrane through which the agent diffuses and the membrane erodes only after its delivery is completed. In this case, drug release is unaffected by the degradation of the matrix.

4. Erosion

Erosion of the coat due to pH and enzymatic hydrolysis causes drug release with certain coat material like glyceryl mono stearate, beeswax and steryl alcohol etc.

1.7 METHOD OF MICROCAPSULE PREPARATION:

(1) Coacervation – phase separation

(2) Interfacial polymerization

(3) In-Situ polymerization

(4) Solvent evaporation

(5) Solvent extraction

(6) Spray drying

(7) Fluidized Bed Coating

(8) MultiorificeCentrifugal process

(9) Pan coating

1. Coacervation – Phase Separation*:* *(Nitika Agnihotri, et al.. 2012)*

Coacervation is a colloid phenomenon. If one starts with a solution of a colloid in an appropriate solvent, then according to the nature of the colloid, various changes can bring about a reduction of the solubility of the colloid. As a result of this reduction a large part of the colloid can be separated out into a new phase. The original one phase system becomes two phases. One is rich and the other is poor in colloid concentration. The colloid-rich phase in a dispersed state appears as amorphous liquid droplets called coacervate droplets. Upon standing these coalesce into one clear homogenous colloid-rich liquid layer, known as the coacervate layer which can be deposited so as to produce the wall material of the resultant capsules.

Coacervation may be initiated in a number of different ways. As the coacervate forms, it must wet the suspended core particles or core droplets and coalesce into a continuous coating for the process of microencapsulation to occur. The final step for microencapsulation is the hardening of the coacervate wall and the isolation of the microcapsules, usually the most difficult step in the total process.

This process of microencapsulation is generally referred to The National Cash Register (NCR) Corporation and the patents of B.K. Green.

This process consists of three Steps-

- Formation of three immiscible phases; a liquid manufacturing phase, a core material phase and a coating material phase
- Deposition of the liquid polymer coating on the core material
- Rigidizing of the coating material

Step-1: The first step of coacervation phase separation involves the formation of three immiscible chemical phases: a liquid vehicle phase, a coating material phase and a core material phase. The three phases are formed by dispersing the core material in a solution of coating polymer, the vehicle phase is used as a solvent for polymer. The coating material phase consists of a polymer in a liquid phase, is formed by using one of the of phase separation- coacervation method, i.e. .by changing the temperature of the polymer solution, by adding a solution, or by inducing a polymer- polymer interaction.

Step-2: It involves the deposition of the liquid polymer coating upon the core material. This is done by controlled mixing of liquid coating material and the core material in the manufacturing vehicle.

Step-3: In the last step rigidizing of the coating material done by the thermal, cross linkingdesolvationtechniques.

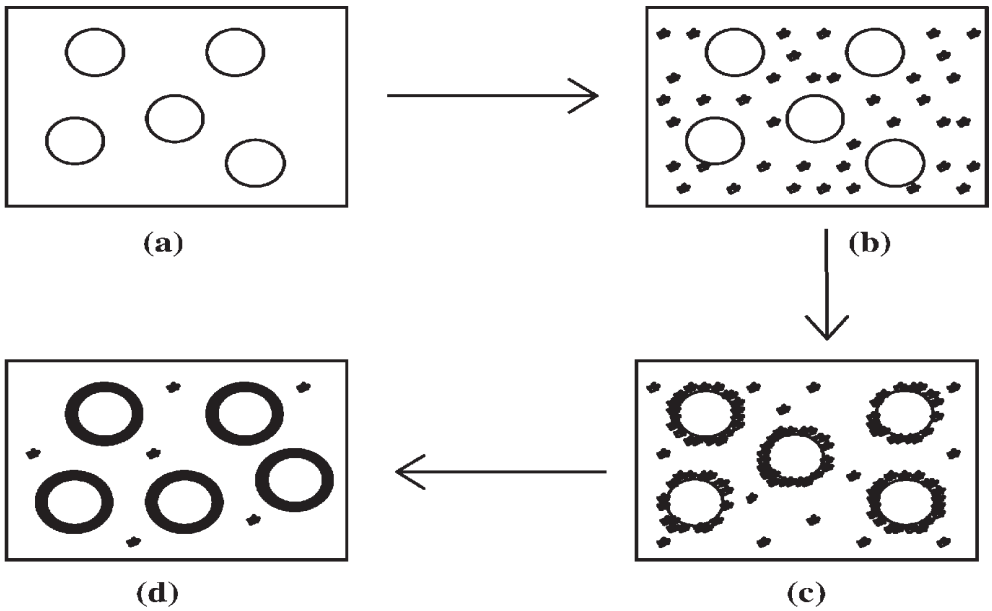


Fig 1.4: Coacervation process: (a) Core material dispersion in solution of shell polymer; (b) Separation of coacervate from solution; (c) Coating of core material by micro droplets of coacervate; (d) Coalescence of coacervate to form continuous shell around core particles.

Simple coacervation

Simple coacervation involves the use of either a second more-water soluble polymer or an aqueous non-solvent for the gelatin. This produces the partial dehydration/desolvation of the gelatin molecules at a temperature above the gelling point. This results in the separation of a liquid gelatin-rich phase in association with an equilibrium liquid (gelatin-poor) which under optimum separation conditions can be almost completely devoid of gelatin. Simple coacervation can be effected either by mixing two colloidal dispersions, one having a high affinity for water, or it can be induced by adding a strongly hydrophilic substance such as alcohol or sodium sulfate [14]. The water soluble polymer is concentrated in water by the action of a water miscible, non-solvent for the emerging polymer (gelatin) phase. Ethanol, acetone,

dioxane, isopropanol and propanol have been used to cause separation of coacervate of gelatin, polyvinyl alcohol and methyl cellulose. Phase separation can be effected by the addition of an electrolyte such as an inorganic salt to an aqueous solution of a polymer such as gelatin, polyvinyl alcohol or carboxymethyl cellulose. A typical simple coacervation using gelatin colloid is as follows: to a 10 percent dispersion of gelatin in water, the core material is added with continuous stirring and at a temperature of 40°C. Then a 20 percent sodium sulfate solution or ethanol is added at 50 to 60 percent by final total volume, in order to induce the coacervation. This system is cooled to 50°C; then, it is necessary to insolubilize the coacervate capsules suspended in the equilibrium liquid by the addition of a hardening agent such as glutaraldehyde and adjusting the pH. The resulting microcapsules are washed, dried and collected

2. Interfacial Polymerization (Ift):

In this method the capsule shell is formed at or on the surface of a droplet or particle by polymerization of reactive monomers.

If the microencapsulating core is water-immiscible liquid then a multifunctional monomer is dissolved in the core material. This solution is dispersed in an aqueous phase containing dispersing agent. A co-reactant is then added to the aqueous phase. This produces a rapid polymerization reaction at the interface which generates the capsule shell.

Advantage: It is a versatile technology able to encapsulation a wide range of core materials, including aqueous solutions, water immiscible liquids and solids.

Disadvantage:

1. Because one of the reactants used to create the capsule shell is dissolved in the core material and is free to react with any groups located on core material molecules to create new molecules.
2. Capsule shell is not uniformly deposited around the core.

3. In situ polymerization:

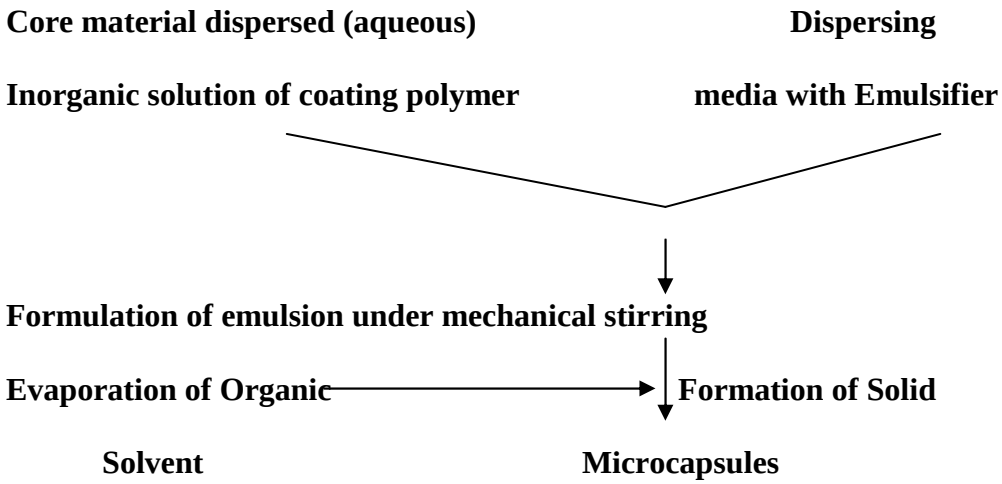
In a few microencapsulation processes, the direct polymerization of a single monomer is carried out on the particle surface. In one process, E.g. Cellulose fibers are encapsulated in polyethylene while immersed in dry toluene. Usual deposition rates are about 0.5μm/min. Coating thickness ranges 0.2-75μm. The coating is uniform, even over sharp projections [27].

4. Solvent-Evaporation Method : **(***Hammad Umar, et al.. 2011*)

(Emulsification- Evaporation Method)

This technique is based on the evaporation of the internal phase of an emulsion by agitation. Initially, the coating polymeric material is dissolved in a volatile organic solvent. The core to be encapsulated is then dispersed in the coating polymer solution to form a suspension or emulsion.

In the next step, this organic solution is emulsified under agitation in dispersing phase, which is immiscible with the organic solvent, which contains the emulsifier. Once the emulsion is stabilized, agitation is maintained and the solvent evaporates after diffusing through the continuous phase. This results in the formation of microcapsule. On the completion of the process, the microcapsules held in suspension in the continuous phase are recovered by filtration or centrifugation and are washed and dried.



Solvent evaporation technique is basically divided into 3 different types of techniques

(I) Oil in water emulsion.

(II)Multiple emulsions: w/o/w:

Advantage: This process is more effective when the water solubility of the drug is high and partitioning between the organic phases is disfavourable.

Application: This process is used for encapsulation of the drugs with weak dose and which are strongly water soluble.

Mechanism of solvent evaporation method:

This system is characterized by the existence of several interfaces through which mass transfer occurs during particle formation, as shown in the below figure:

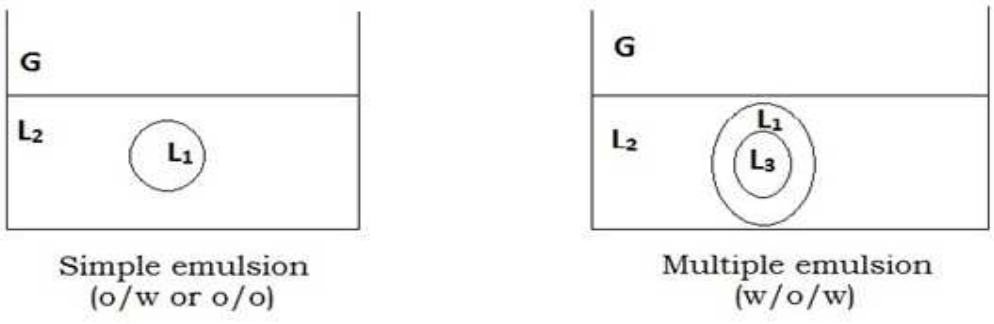


Fig 1.5: Mechanism of solvent evaporation method

Organic solvent of the dispersed phase of the emulsion is eliminated in two stages:

1. Diffusion of the solvent in the dispersing phase.
2. Elimination of the solvent at dispersing phase – air interface.

The formation of solid microcapsule is brought about by the evaporation of the volatile solvent L_1 at interface L_2/G. During the course of solvent evaporation, a partitioning is produced across the interface L_1/L_2 from the dispersed phase to continuous phase leading to the formation of solid microcapsules.

5. Solvent – Extraction method:

As mentioned in the previous method, the organic solvent of the dispersed phase of the emulsion is eliminated in two stages i.e.

i. Diffusion into continuous phase &
ii. Elimination of solvent at continuous phase – air interface.

If one uses a continuous phase which will immediately extract the solvent of the dispersed phase, the evaporation stage is no longer necessary in microencapsulation.

In practice it is achieved

a. By using large volume of dispersing phase w.t.o dispersed phase.
b. By choosing a co-solvents in dispersed phase, of which at least one has a great affinity for the dispersing phase.
c. By formulating a dispersing phase with two solvents in which one acts as a solvent extractor of the dispersed phase.

6. Spray–drying *(Nitika Agnihotri, et al.. 2012)*

Spray drying serves as a microencapsulation technique when an active material is dissolved or suspended in a melt or polymer solution and becomes trapped in the dried particle. Coating solidification in the case of spray drying is effected by rapid evaporation of a solvent in which the coating material is dissolved. Coating solidification in spray congealing methods, however, is accomplished by thermally congealing a molten coating material or by solidifying a dissolved coating by introducing the coating - core material mixture into a nonsolvent. Removal of the nonsolvent or solvent from the coated product is then accomplished by sorption, extraction, or evaporation techniques. In practice, microencapsulation by spray drying is conducted by dispersing a core material in a coating solution, in which the coating substance is dissolved and in which the core material is insoluble, and then by atomizing the mixture into air stream. The air, usually heated, supplies the latent heat of vaporization required to remove the solvent from the coating material, thus forming the microencapsulated product[21]. The equipment components of a standard spray dryer include an air heater, atomizer, main spray chamber, blower or fan, cyclone and product collector. Microencapsulation by spray congealing can be accomplished with spray drying equipment when the protective coating is applied as a melt. Coating solidification (and microencapsulation) is accomplished by spraying the hot mixture into a cool air stream. Waxes, fatty acids and alcohols, polymers and sugars, which are solids at room temperature but meltable at reasonable temperatures, are applicable to spray congealing techniques. Typically, the particle size of spray congealed products can be accurately controlled when spray drying equipment is used, and has been found to be a function of the feed rate, the atomizing wheel velocity, dispersion of feed material viscosity, and variables [24].

Advantage: Low cost of encapsulation and able to produce large amount of microcapsules.

Disadvantage: This process is limited to coating material soluble in water, but the list of water soluble coating materials are limited.

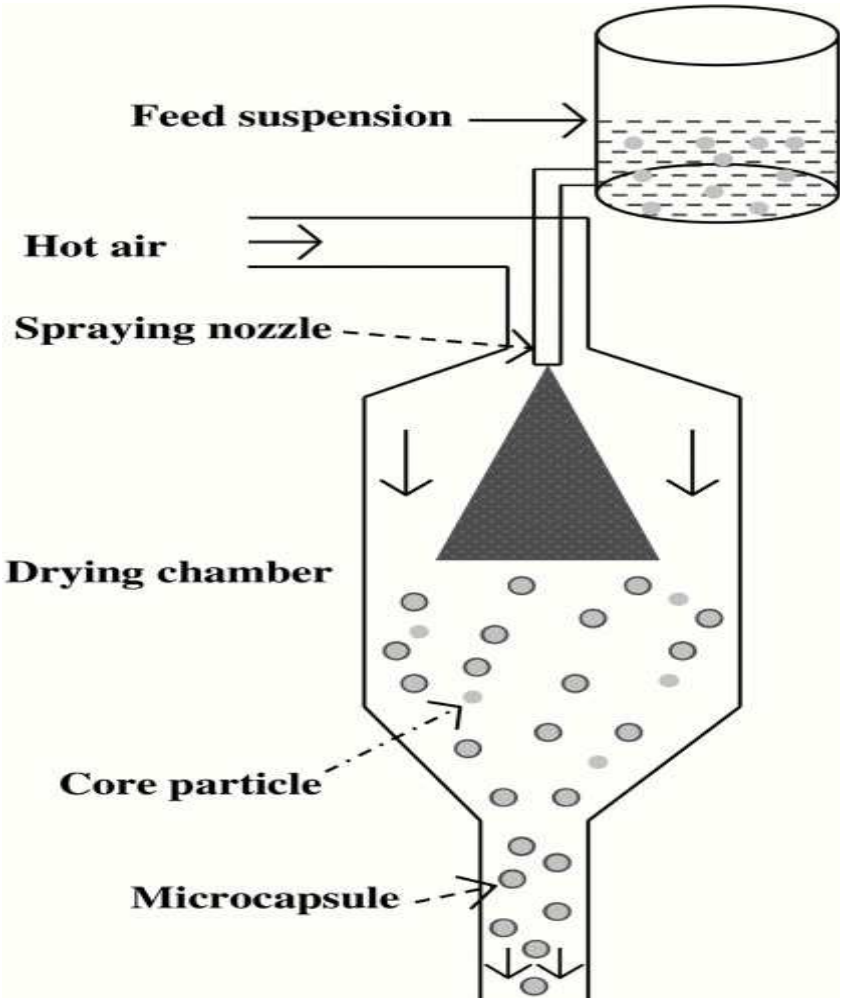


Fig. 1.6: Spray Dryer

7. Fluidized bed coating (Wurster Air Suspension):

It consists of the dispersing of solid core material in a supporting air steam and then spray coating of the air suspended particles.

Advantage: Able to handle an extremely wide range of coating formulations.

8. Multi-orifice – Centrifugal processes:

In this process it utilizes centrifugal forces to hurl a core material particle through an enveloping microencapsulating membrane, there by effecting mechanical microencapsulation.

9. Pan coating *(Nitika Agnihotri, et al.. 2012)*

In this pan coating the particles are tumbled in a pan or other device while the coating material is applied slowly[17].

The particles are tumbled in a pan or other device while the coating material is applied slowly with respect to microencapsulation, solid particles greater than 600 microns in size are generally considered essential for effective coating, and the process has been extensively employed for the preparation of controlled-release beads. Medicaments are usually coated onto various spherical substrates such as nonpareil sugar seeds, and then coated with protective layers of various polymers.

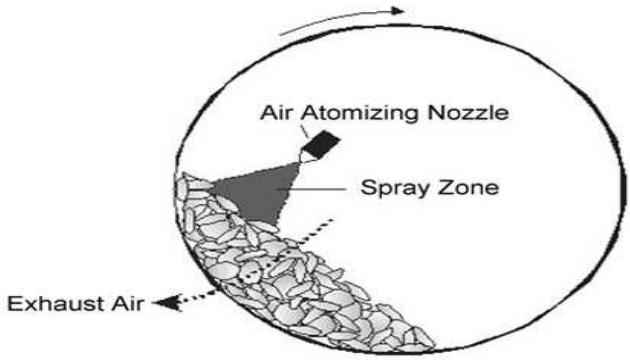


Fig 1.7: Representation of a typical pan coating

Usually, to remove the coating solvent, warm air is passed over the coated materials as the coatings are being applied in the coating pans. In some cases, final solvent removal is accomplished in a drying oven.

Strategy for improving Encapsulation efficiency of drug:

i. Water solubility of the drug can be reduced by chemical modification prior to its incorporation in the organic phase. However, such structural modification may give rise to toxicological problems.

ii. Modifying the dispersing phase of the emulsion to reduce leakage of the drug from the oily droplets of polymer solution. Modifications like,

   a. Saturating the continuous phase with the drug.

   b. Adjusting the pH of this same phase

   c. Adding the electrolytes.

1.8 POLYMERS USED FOR MICROENCAPSULATION:

(*Hammad Umar, et al..2011)*

(I) Water soluble resins

(1) Gelatin

(2) Gum Arabia

(3) Starch

(4) Polyvinyl pyrrolidone

(5) Sodium carboxy methyl cellulose

(6) Hydroxy ethyl cellulose

(7) Mehtyl cellulose

(8) Arabinogalactam

(9) Polyvinyl alcohol

(10) Polyacrylic acid

(II) Water insoluble resins

(1) Ethyl cellulose

(2) Polmethyl methacrylate (PMMA)

(3) Polymethacrylate (Eudragit)

(4) Polyethylene

(5) Polyamide (Nylon)

(6) Poly (Ethylene-Vinyl acetate)

(7) Cellulose nitrate

(8) Silicones

(9) Poly (lactide-co-glycolide)

(10) Cellulose acetate butyrate

(III) Waxes & Lipids

1. Paraffin
2. Carnauba Wax
3. Spermaceti
4. Bees wax
5. Stearic acid
6. Strearyl alcohol
7. Glyceryl stearates

(IV) Enteric Resins

1. Shellac
2. Cellulose acetate phthalate
3. Zein

1.9 Application of microencapsulation. *(Nitika Agnihotri, et al.. 2012)*

There are many reasons why drugs and related chemicals have been microencapsulated. The technology has been used widely in the design of controlled release and sustained release dosage forms.

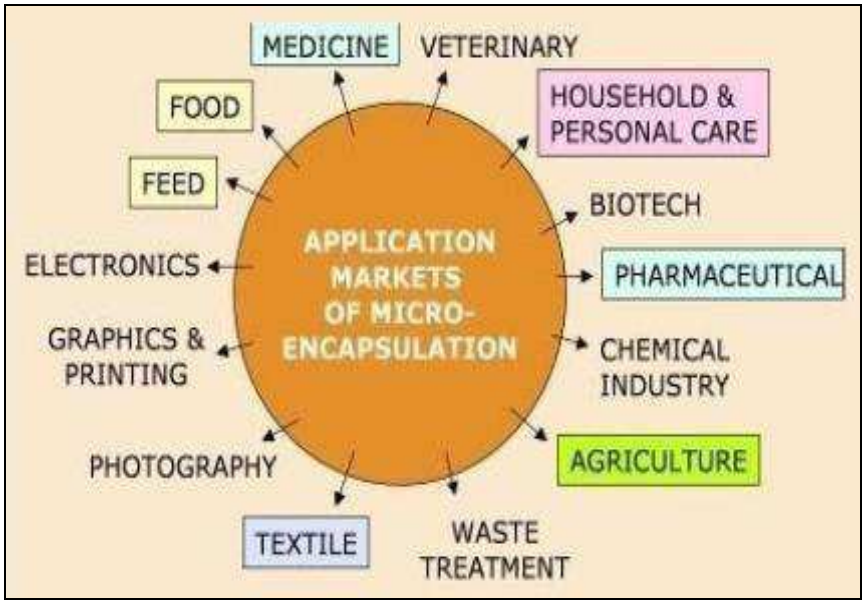


Fig.1. 8: Applications of microencapsulation.

- To mask the bitter taste of drugs like Paracetamol, Nitrofurantoin etc.
- Many drugs have been microencapsulated to reduce gastric and other G.I. tract irritations. Sustained release Aspirin preparations have been reported to cause significantly less G.I. bleeding than conventional preparations.
- A liquid can be converted to a pseudo-solid for easy handling and storage.
  e.g. Eprazinone.
- Hygroscopic properties of core materials may be reduced by microencapsulation e.g. Sodium chloride.
- Carbon tetra chlorides and a number of other substances have been microencapsulated to reduce their odor and volatility.
- Microencapsulation has been employed to provide protection to the core materials against atmospheric effects, e.g. vitamin A.

- Separation of incompatible substance has been achieved by encapsulation.
- Cell immobilization: In plant cell cultures, Human tissue is turned into bio-artificial organs, in continuous fermentation processes.
- Protection of molecules from other compounds.
- Drug delivery: Controlled release delivery systems.
- Quality and safety in food, agricultural & environmental sectors.
- Beverage production, Soil inoculation.
- In textiles**:** means of imparting finishes.
- Protection of liquid crystals.

AIM &

OBJECTIVES....

2. AIM AND OBJECTIVES

Cancer is a leading cause of death world wide. More than 70% of all cancer deaths occurred in low and middle-income countries. Deaths from cancer world wide are projected to continue rising, with an estimated 12 million deaths in 2030.

Treatment of cancer includes chemotherapy, radiation therapy, gene therapy, photodynamic therapy, biologic therapy, surgical removal of tumor cells, etc. Cancer treatments vary according to the type of cancer and the extent of the tumor. Chemotherapy is the most convenient and non-expensive when compared to other modes of treatment. Varieties of anticancer drugs are available in the market and some of them are under clinical trials. The main problem with anti-cancer drugs is that they not only affect the cancerous cells but also affect the normalcells. These happen dueto non-specific targeting to cancerous cells and hence other normal cells get affected.

Recently, drug targeting especially targeting of drugs by microcapsules have been getting much attention by the researchers for treating cancer. Acritical advantage in treating cancer with microcapsules is the inherent leaky vasculature present serving cancerous tissues. The effective vascular architecture, created dueto rapid vascularization necessary to serve fast-growing cancers, coupled with poor lymphatic drainage allows an enhanced permeation and retention effect.

Targeting the tumor vasculature is a strategy that can allow targeted delivery to a wide range of tumor types. Tremendous opportunities exist for using microcapsulesas sustained drug delivery systems for cancer treatment. Natural and synthetic co-polymers including albumin, fibrinogen, alginate, chitosan and collagen have been used forther fabrication of microcapsules.

Objectives:

The objective ofthe present study is preparing the microcapsules of 5-fluorouraccil in order to provide sustained release. The micro capsules of 5-fluorouracil were formulated by coacervation phase separation by change in pH method and emulsion solvent evaporation.The micro capsules is evaluated with respect to particle size, drug content, entrapment efficiency. Drug polymer compatibility studied by FTIR and DSC. In-vitro drug release study, release kinetics studies and stability studies.

PLAN OF WORK....

3. PLAN OF WORK

- **Literature survey**.
- **Materials and equipments.**
- **Preformulation studies.**
  - **Characterization of Drug.**
    - Appearance.
    - Melting Point Determination.
    - Solubility Study.
    - UV Spectroscopy (λ_{max}).
    - IR Spectroscopy.
    - Loss on drying.
  - **Drug – Polymers InteractionStudies.**
    - Fourier transforms Infra-Red (FTIR) Spectroscopy Study.
    - Differential Scanning Calorimetry (DSC) Analysis.
  - **Preparation of 5-fluorouracil microcapsules.**
  - **Evaluation of 5-fluorouracil microcapsules.**
    - Appearance.
    - Particle size.
    - Evaluation of micrcapsules.
    - Content uniformity.
    - Scanning electron microscopy.
    - Invitro drug release studies.

- Release drug data model fitting.

- **Results and Discussion.**
- **Summary and Conclusion.**
- **Future Prospects.**
- **Bibliography.**

LITERATURE SURVEY...

4. LITERATURE SURVEY

2.1. Literature Review:

Alaa Eldeen Bakry Yassin., et al. (2010) The aim of this study was to formulate a new orally-administered colon delivery system of 5-flurouracil (5-FU) for the treatment of colon cancer. The system was designed to target 5-FU directly to the colon with high potential of much more effective and less toxic colon cancer treatment. The system was prepared by compression coating technique using granulated chitosan. The method was optimized by studying the effect of granulation and thickness of the coat with respect to the in vitro performance in a medium mimicking mouth-to-colon environment. The in vivo selectivity of the system was assessed by X-ray imag- ing technique using beagle dogs. Results showed that granulation of chitosan were effective in protecting against the known acid solubility of the polymer. Formula (F7) with coat weight of 50 mg/tablet exhibited the best protection profile with <10% of the drug released after 6 h. The resistance of the system to the simulated gastro-intestinal media was reduced as the chitosan coat weight decreases. The performance of the system in a rat caecal contents containing-medium showed that the susceptibility of this system for the enzymatic degradation by colonic enzymes. The X-ray imaging gave rise to the in vivo selectivity of this system for colon targeting by showing the resistivity of the system to the stomach and small intestine environment and the selective disintegration of the system inside the largebowl.

A.V Yadav., et al.. (2009) Aceclofenac was formulated as novel enteric microcapsules for improved delivery to the intestine using thepolymer ethyl cellulose as the retardant material. Micro encapsulation of Aceclofenac was done to achieve a

controlled drugrelease profile suitable for per oral administration. Aceclofenac was used as core and microcapsules were prepared by anemulsion solvent evaporation method. The prepared microcapsules were evaluated for size analysis, drug content,encapsulation efficiency, wall thickness, optical microscopy and drug release characteristics. All microcapsules obtained werediscrete, large sized, free flowing and spherical in shape. Aceclofenac release from microcapsules followed higuchi model andinfluenced by the size of the microcapsules. Slow release of Aceclofenac from ethyl cellulose microcapsules over 12 hour's was observed.

Krishnaiah YS, Satyanarayana V., et al. (2012)

Intravenous administration of 5-fluorouracil for colon cancer therapy produces severe systemic side-effects due to its cytotoxic effect on normal cells. The broad objective of the present study was to develop novel tablet formulations for site-specific delivery of 5-fluorouracil to the colon without the drug being released in the stomach or small intestine using guar gum as a carrier. Fast-disintegrating 5-fluorouracil core tablets were compression coated with 60% (FHV-60), 70% (FHV-70) and 80% (FHV-80) of guar gum, and were subjected to in vitro drug release studies. The amount of 5-fluorouracil released from the compression-coated tablets in the dissolution medium at different time intervals was estimated by a HPLC method. Guar gum compression-coated tablets released only 2.5-4% of the 5-fluorouracil in simulated GI fluids. When the dissolution study was continued in simulated colonic fluids (4% w/v rat caecal content medium) the compression-coated FHV-60, FHV-70 and FHV-80 tablets released another 70, 55 and 41% of the 5-fluorouracil respectively. The results of the study show that compression-coated tablets containing 80% (FHV-80) of guar gum are most likely to provide targeting of 5-fluorouracil for local action in the colon, since they released only 2.38% of the drug in the physiological environment of the

stomach and small intestine. The FHV-80 formulation showed no change either in physical appearance, drug content or dissolution pattern after storage at 40 degrees C/RH 75% for 6 months. The differential scanning calorimetric study showed that 5-fluorouracil did not interact with the formulation excipients used in the study.

Shaik. Shabbeer., et al. (2010)

The present work describes the preparation of Sodium alginate/chitosan microcapsules containing 5-Fluorouracil (5-FU) intended for colon-specific delivery. The alginate/chitosan micro granules were prepared by the cross linking technique with calcium chloride(4%). Microscopy and Digital photography was used for morphology observation, which shows spherical shape but rough surface of the micro particles. Transmission infrared spectra of chitosan powder, 5-fluorouracil, sodium alginate pectin, and prepared microcapsules were acquired to draw information on the molecular state of chitosan and 5-fluorouracil. Differential scanning calorimetry (DSC) studies of 5-Fluorouracil, Chitosan, sodium alginate and pectin, were performed with PerkinElmer Thermal Analysis (Mettler Toledo 821Thermal analyzer) Calibrated with indium as standard. For thermogram acquisition The drug content and release profile of 5-FU was determined by UV–Vis absorption measurement at λ max 266 nm. The drug content was found to be 0.061mg of 5-FU /mg of alginate/chitosan microcapsules. The swelling behavior and release of drug was determined at two different pH conditions i.e. at pH 1.2 and pH 6.8. micro particles were swelling but did not dissolves give more sustain manner of release. In order to study the effect of alginate on drug release from microcapsules. Accordingly, three different batches (F1, F2 & F3) containing 1.5% w/v, 2.0% and 2.5% w/v of alginate based microcapsules were prepared (batches A1,A2 and A3). The results of in vitro studies shown 5-fluorouracil remain intact and shows minimal drug release in stomach and

small intestine, it is very advantage because 5-fluorouracil the initial release it is required to be drastically minimized to avoid the sight effects associated with these agents.

Ujwala A Shinde, Mangal S Nagarsenker., et al. (2009) A gelatin and sodium alginate complex coacervation system was studied and an effect of pH and colloid mixing ratios on coacervation was investigated. A colloid mixing ratio at which optimum coacervation occurred varied with the coacervationpH.Viscometric, turbidity and coacervate dry yield investigations were used to investigate optimum conditions for complex coacervation. Optimum coacervation occurred at pH 3.5 at a gelatin sodium alginate ratio 4:1. Coacervate and equilibrium fluid was analyzed for gelatin and sodium alginate contents and yields calculated on the basis of chemical analysis showed that optimum coacervation occurred 0at 25% sodium alginate fraction at pH 3.5.

SONIA GUPTA, PMS BEDI, NEENA BEDI., et al. (2010)

Various formulations of pectin matrix tablets containing 5Fluorouracil (5FU) coated with combination of Eudragit RS100 and inulin were prepared and evaluated for release of drug in the colon, which is a prerequisite for the effective treatment of colorectal carcinoma. In vitro dissolution studies of formulations F1, F2, F3 and F4 containing 30%, 45%, 60% and 75% by weight of pectin respectively revealed that formulations F1, F2, F3 and F4 released the entire drug after 3, 4, 6 and 11 hours of the study. The cumulative percentage release data of formulations F5, F6, F7, F8 and F9 containing 75% by weight of pectin in the matrix coated with combination of Eudragit RS 100 and inulin in the ratio of 100%, 90%: 10%, 80%: 20%, 70%: 30% and 60%: 40% revealed that F9 is the best formulation as it released only 13.2±3.21% of drug after 5 hours. To further retard the initial release, formulations F10 and F11

were coated to obtain a weight gain of 10.85% and 12.91% of total weight of tablets respectively. Formulation F11 showed the best release data as it released 8.5±2.58% of drug after 5 hrs, which was less than that released by other formulations. The comp lete drug release from formulation F11 was further tested in vitro in the presence of rat caecal contents and it was observed that 87.1±3.5% of drug was released after 24 hrs in the presence of rat caecal content.

Vaghani SS, JivaniNP., et al. (2011)

In the present investigation, 5-fluorouracil loaded microspheres of Eudragit (RS 100, RL 100 and RSPO) and ethylcellulose were prepared. "O/O solvent evaporation" technique was used for preparation of microspheres using (methanol + acetone)/liquid paraffin system. Magnesium stearate was used as the droplet stabilizer and n-hexane was added to harden the microspheres. The prepared microspheres were characterized for their micromeretic properties and entrapment efficiency; as well by Fourier transform infrared spectroscopy (FTIR) and thin layer chromatography (TLC). Photomicrographs were taken to study the shape of microspheres. The best fit release kinetics was achieved with Higuchi plot. Mean particle size, entrapment efficiency and production yields were highly influenced by the type of polymer and polymer concentration. It is concluded from the present investigation that various Eudragit and Ethylcellulose are promising controlled release carriers for 5-FU.

Ziyaur Rahman, KanchanKohli.et al (2006)

The purpose of this investigation was to prepare and evaluate the colon-specific microspheres of 5-fluorouracil for the treatment of colon cancer. Core microspheres of alginate were prepared by the modified emulsification method in liquid paraffin and by cross-linking with calcium chloride. The core microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the

stomach and small intestine. The microspheres were characterized by shape, size, surface morphology, size distribution, incorporation efficiency, and in vitro drug release studies. The outer surfaces of the core and coated microspheres, which were spherical in shape, were rough and smooth, respectively. The size of the core microspheres ranged from 22 to 55 µm, and the size of the coated microspheres ranged from 103 to 185 µm. The core microspheres sustained the drug release for 10 hours. The release studies of coated microspheres were performed in a pH progression medium mimicking the conditions of the gastrointestinal tract. Release was sustained for up to 20 hours in formulations with core microspheres to a Eudragit S-100 coat ratio of 1:7, and there were no changes in the size, shape, drug content, differential scanning calorimetrythermogram, and in vitro drug release after storage at 40°C/75% relative humidity for 6 months.

DRUG AND POLYMER

PROFILE

4.2. DRUGPROFILE

(Indian Pharmacopoeia, 2007*)*

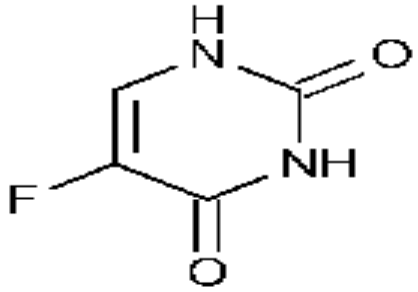
(www.Fluorouracil - Wikipedia, the free encyclopedia.html)

(www.fluorouracil/DrugBank Fluorouracil (DB00544).html)

Fluorouracil is a pyrimidine analog that is an antineoplastic ant metabolite. It interferes with DNA synthesis by blocking the thymidylate synthetase conversion of deoxyuridylic acid to thymidylic acid.

DRUG NAME: 5- Fluorouracil

Molecular structure:



Molecular formula: $C_4H_3FN_2O_2$.

Molecular weight: 130.077 g/mol.

IUPAC Name:5-fluoro-1H-pyrimidine-2, 4-dione...

CAS NUMBER: [*51-21-8*].

Melting point: 282-286°c

Physical properties:

It is a white crystalline powder, odorless in nature. Soluble in water, partially soluble in cold water, methanol and soluble in diethyl ether.

Mechanism of action:

The precise mechanism of action has not been fully determined, but the main mechanism of fluorouracil is thought to be the binding of the deoxyribonucleotide of the drug (FdUMP) and the folate cofactor, N5–10-methylenetetrahydrofolate, to thymidylate synthase (TS) to form a covalently bound ternary complex. This results in the inhibition of the formation of thymidylate from uracil, which leads to the inhibition of DNA and RNA synthesis and cell death. Fluorouracil can also be incorporated into RNA in place of uridine triphosphate (UTP), producing a fraudulent RNA and interfering with RNA processing and protein synthesis.

Pharmacokinetics:

Absorption: 28-100%

Distribution: Into all body water by passive diffusion, crosses placenta, BBB, high and persistent levels in malignant effusions.

Protein binding: 8 to12%

Metabolism: Hepatic metabolism.

Excretion:

Seven percent to 20% of the parent drug is excreted unchanged in the urine in 6 hours; of this over 90% is excreted in the first hour. The remaining percentage of the administered dose is metabolized, primarily in the liver.

Half life: 10 to 20 min.

Indications:

5-fluorouracil is one of the oldest and best antineoplastic drug. For more than four decades 5-fluorouracil has been widely used in the therapy of different solid tumor types namely cancer of the stomach, liver, intestine, pancreas, ovary, breast alone or in combination chemotherapy regimes it is one of the most used for the

treatment of colorectal cancer, 5-fluorouracil has been in use against cancer for about 40 years. It is used in treating colorectal cancer, and pancreatic cancer, in which it has been the established form of chemotherapy for decades. It is also sometimes used in the treatment of inflammatory breast cancer. 5-FU is also used in ophthalmic surgery, specifically to augment trabeculectomy (an operation performed to lower the intraocular pressure in patients with glaucoma). Fluorouracil can be used topically for the treating actinic (solar) keratoses and some types of basal cell carcinomas of the skin.

Adverse effects:

Diarrhea, nausea, and possible occasional vomiting mouth sores, poor appetite, watery eyes, taste changes, discoloration along vein through which medication is given and low blood counts temporarily.

Adverse reactions include chest pain, ECG changes and increase in cardiac enzymes which may indicate problems with the heart. These symptoms are very rare but increased for patients with a prior history of heart disease.

Most people do not experience all of the side effects listed. Side effects are often predictable in terms of their onset and duration and are almost always reversible and will disappear after treatment is complete.

4.3 POLYMERSPROFILE

4.3.1 GELATIN

Nonproprietary Names:

BP : Gelatin

JP : Gelatin

PhEur : Gelatin

USP-NF : Gelatin

Synonyms: gelatina; gelatine; Instagel; Kolatin; Solugel; Vitagel.

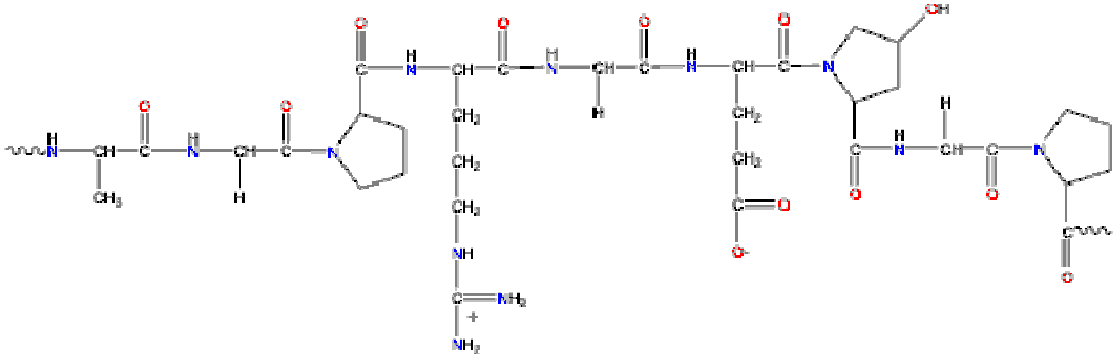
Chemical Name: Gelatin

CAS Registry Number: [9000-70-8]

Empirical Formula and Molecular Weight:

Gelatin is a generic term for a mixture of purified protein fractions obtained either by partial acid hydrolysis (type A gelatin) or by partial alkaline hydrolysis (type B gelatin) of animal collagen Obtained from cattle and pig bone, cattle skin (hide), pigskin, and fish skin. Gelatin may also be a mixture of both types. The protein fractions consist almost entirely of amino acids joined together by amide linkages to form linear polymers, varying in molecular weight from 20,000–200,000.

Structural Formula:



Functional Category:

Coating agent, film-forming agent, gelling agent, suspending agent, tablet binder and viscosity-increasing agent.

Description:

Gelatin occurs as a light-amber to faintly yellow-colored, vitreous, brittle solid. It is practically odorless and tasteless, and is available as translucent sheets, flakes, and granules, or as a coarse powder.

Color **:** light amber to faintly yellow coloured.

Odor **:** odorless.

Taste **:** Tasteless

Texture **:** brittle solid

Acidity / Alkalinity: For a 1% w/v aqueous solution at 258C (depending on source And grade)

pH = 3.8–5.5 (type A);

pH = 5.0–7.5 (type B).

Solubility:

Practically insoluble in acetone, chloroform, ethanol (95%), ether, and methanol. Soluble in glycerin, acids, and alkalis, although strong acids or alkalis cause precipitation. In water, gelatin swells and softens, gradually absorbing between five and 10 times its own weight of water. Gelatin is soluble in water above 408ºC, forming a colloidal solution, which gels onCooling to 35–408ºC. This gel–sol system is thixotropic and heatreversible, the melting temperature being slightly higher than the setting point; the melting point can be varied by the addition of glycerin.

Stability and StorageConditions:

Dry gelatin is stable in air. Aqueous gelatin solutions are also stable for long periods if stored under cool conditions but they are subject to bacterial degradation. At temperatures above about 508ºC, aqueous gelatin solutions may undergo slow depolymerization and a reduction in gel strength may occur on resetting. Depolymerization becomes more rapid at temperatures above 658ºC, and gel strength may be reduced by half when a solution is heated at 808ºC for 1 hour. The rate and extent of depolymerization depends on the molecular weight of the gelatin, with a lower-molecular-weight material decomposing more rapidly.Gelatin may be sterilized by dry heat. The bulk material should be stored in an airtight container in a cool, well-ventilated and dry place.

Incompatibilities:

Gelatin is an amphoteric material and will react with both acids and bases. It is also a protein and thus exhibits chemical properties characteristic of such materials; for example, gelatin may be hydrolyzed by most proteolytic systems to yield its amino acid components. Gelatin will also react with aldehydes and aldehydic sugars, anionic and cationic polymers, electrolytes, metal ions, plasticizers, preservatives, strong oxidizers, and surfactants. It is precipitated by alcohols, chloroform, ether, mercury salts, and tannic acid. Gels canbe liquefied by bacteria unless preserved. Some of these interactions are exploited to favorably alter the physical properties of gelatin: for example, gelatin is mixed with aplasticizer, such as glycerin, to produce soft gelatin capsules and suppositories; gelatin is treated with formaldehyde to produce gastro resistance.

4.3.2 SODIUM ALGINATE

Nonproprietary Names:

BP : Sodium Alginate

PhEur : Sodium Alginate

USP-NF : Sodium Alginate

Synonyms:

Alginatosodico, algin, alginic acid, sodium salt, E401, Kelcosol, Keltone, natriialginas, Protanaland sodiumpolymannuronate.

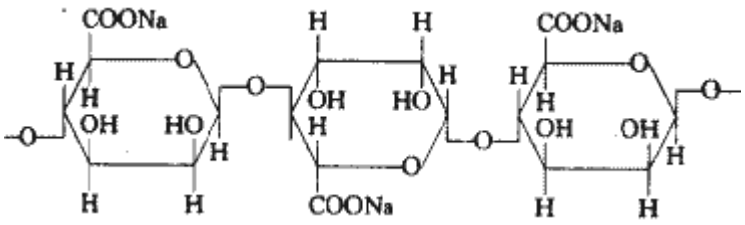
Chemical Name: Sodium alginate

CAS Registry Number: [9005-38-3]

Empirical Formula and Molecular Weight:

Sodium alginate consists chiefly of the sodium salt of alginicacid, which is a mixture of polyuronic acids composed of residues of Dmannuronicacid and L-guluronic acid.

Structural Formula:



Functional Category:

Stabilizing agent, suspending agent, tablet and capsule disintegrant.

Description:

Sodium alginate occurs as an odorless and tasteless, white to pale yellowish-brown colored powder.

Color **:** pale yellowish-brown

Odor **:** odorless.

Taste **:** tasteless

Texture : powder

Acidity/alkalinity: pH _ 7.2 (1% w/v aqueous solution)

Solubility:

Practically insoluble in ethanol (95%), ether, chloroform, and ethanol/water mixtures in which the ethanol content is greater than 30%. Also, practically insoluble in other organic solvents and aqueous acidic solutions in which the pH is less than 3. Slowly soluble in water, forming a viscous colloidal solution.

Stability and Storage Conditions:

Sodium alginate is a hygroscopic material, although it is stable if stored at low relative humidities and a cool temperature. Aqueous solutions of sodium alginate are most stable at pH4–10. Below pH 3, alginic acid is precipitated. A 1% w/v aqueous solution of sodium alginate exposed to differing temperatures had a viscosity 60–80% of its original value after storage for 2 years. Solutions should not be stored in metal containers. Sodium alginate solutions are susceptible on storage to microbial spoilage, which may affect solution viscosity. Solutions are ideally sterilized using ethylene oxide, although filtration using a 0.45 mm filter also has only a slight adverse effect on solution viscosity. Heating sodium alginate solutions to temperatures above 70°C causes depolymerization with a subsequent loss of viscosity. Autoclaving of solutions can cause a decrease in viscosity, which may vary depending upon the nature of any other substances present. Gamma irradiation should not be used to sterilize sodium alginate solutions since this process severely reduces solution viscosity. Preparations for external use may be preserved by the addition of 0.1% chlorocresol, 0.1% chloroxylenol, or parabens. If the medium is acidic, benzoic acid may also be used. The bulk material should be stored in an airtight container in a cool, dry place.

Incompatibilities:

Sodium alginate is incompatible with acridine derivatives, crystal violet, phenyl mercuric acetate and nitrate, calcium salts, heavy metals, and ethanol in concentrations greater than 5%. Low concentrations of electrolytes cause an increase in viscosity but high electrolyte concentrations cause salting-out of sodium alginate; salting-out occurs if more than 4% of sodium chloride is present.

Applications in Pharmaceutical Formulation or Technology:

1. Sodium alginate is used in a variety of oral and topical pharmaceutical formulations.
2. In tablet formulations, sodium alginate may be used as both a binder and disintegrant; it has been used as a diluent in capsule formulations.
3. Sodium alginate has also been used in the preparation of sustained-release oral formulations since it can delay the dissolution of a drug from tablets, capsules and aqueous suspensions.
4. The effects of particle size, viscosity and chemical composition of sodium alginate on drug release from matrix tablets have been described.
5. In topical formulations, sodium alginate is widely used as a thickening and suspending agent in a variety of pastes, creams, and gels, and as a stabilizing agent for oil-in-water emulsions.
6. Recently, sodium alginate has been used for the aqueous microencapsulation of drugs, in contrast with the more conventional microencapsulation techniques which use organic solvent systems. It has also been used in the formation of nanoparticles
7. Sodium alginate is also used in cosmetics and food products

Table 4.1: Uses of sodium alginate.

USE	**CONCENTRATION (%)**
Pastes and creams	5-10
Stabilizer in emulsions	1-3
Suspending agent	1-5
Tablet binder	1-3
Tablet disintigrent	2.5-10

4.3.3 ETHYL CELLULOSE

Nonproprietary Names:

BP : Ethyl cellulose

PhEur : Ethyl cellulose

USP-NF : Ethyl cellulose

Synonyms: Aquacoat ECD, Aqualon, Ashacel, E462, Ethocel, ethylcellulosum Surelease.

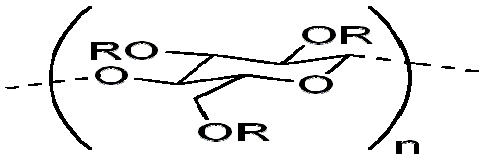
Chemical Name: Cellulose ethyl ether.

CAS Registry Number: [9004-57-3]

Empirical Formula and Molecular Weight:

Ethyl cellulose is partially ethoxylated. Ethyl cellulose with complete ethoxyl substitution (DS = 3) is $C_{12}H_{23}O_6$ ($C_{12}H_{22}O_5$) $nC_{12}H_{23}O_5$ where n can vary to provide a Wide variety of molecular weights. Ethyl cellulose, an ethyl ether of cellulose, is a long-chain polymer of b- anhydroglucose units joined together by acetal linkages.

Structural Formula:



R = H or CH_2CH_3

Functional Category:

Coating agent, flavoring agent, tablet binder, tablet filler,viscosityincreasing agent.

Description:

Ethyl cellulose is a tasteless, free-flowing, and white to light tan-colored powder.

Color **:** white to light tan-colored powder

Odor **:** odorless.

Taste **:** tasteless

Texture **:** powder

Solubility:

Ethyl cellulose is practically insoluble in glycerin, propylene glycol, and water. Ethyl cellulose that contains lessthan 46.5% of ethoxyl groups is freely soluble in chloroform, methyl acetate, and tetrahydrofuran, and in mixtures ofaromatic hydrocarbons with ethanol (95%). Ethyl cellulosethat contains not less than 46.5% of ethoxyl groups is freely soluble in chloroform, ethanol (95%), ethyl acetate, methanol, and toluene.

Stability and Storage Conditions:

Ethyl cellulose is a stable, slightly hygroscopic material. It ischemically resistant to alkalis, both dilute and concentrated, andto salt solutions, although it is more sensitive to acidic materialsthan are cellulose esters.Ethyl cellulose is subject to oxidative degradation in the presenceof sunlight or UV light at elevated temperatures. This may beprevented by the use of antioxidant and chemical additives thatabsorb light in the 230–340nm range.Ethyl cellulose should be stored at a temperature not exceeding 328ºC (908F) in a dry area away from all sources of heat. It shouldnot be stored next to peroxides or other oxidizing agents.

Incompatibilities:

Incompatible with paraffin wax and microcrystalline wax.

Applications in Pharmaceutical Formulation or Technology

- Ethyl cellulose is widely used in oral and topical pharmaceutical formulations.
- The main use of ethyl cellulose in oral formulations is as a hydrophobic coating agent for tablets and granules. Ethyl cellulose coatings are used to modify the release of a drug, to mask an unpleasant taste, or to improve the

stability of a formulation. For example where granules are coated with ethyl cellulose to inhibit oxidation.

- Modified-release tablet formulations may also beProduced using ethyl cellulose as a matrix former. Ethyl cellulose, dissolved in an organic solvent or solvent mixture, can be used on its own to produce water-insoluble films.
- Drug release through ethyl cellulose-coated dosage forms can be controlled by diffusion through the film coating. This can be a slow process unless a large surface area (e.g. capsules or granules compared with tablets) is utilized. In those instances, aqueous ethyl cellulose dispersions are generally used to coat granules or capsules.
- Ethyl cellulose-coated beads and granules have also demonstrated the ability to absorb pressure and hence protect the coating from Fracture during compression.
- High-viscosity grades of ethyl cellulose are used in drug microencapsulation.
- Release of a drug from an ethyl cellulose microcapsule is a function of the microcapsule wall thickness and surface area.
- In tablet formulations, ethyl cellulose may additionally be employed as a binder, the ethyl cellulose being blended dry or wet granulated with a solvent such as ethanol (95%).
- Ethyl cellulose produces hard tablets with low friability, although they may demonstrate poor dissolution. Ethyl cellulose has also been used as an agent for delivering therapeutic agents from oral (e.g. dental) appliances.
- In topical formulations, ethyl cellulose is used as a thickening agent in creams, lotions, or gels, provided an appropriate solvent is used. Ethyl cellulose has

been studied as a stabilizer for emulsions. Ethyl cellulose is additionally used in cosmetics and food products.

Table 4.2: Uses of ethyl cellulose.

Use	**Concentration (%)**
Microencapsulation	10.0–20.0
Sustained-release tablet coating	3.0–20.0
Tablet coating	1.0–3.0
Tablet granulation	1.0–3.0

MATERIALS & EQUIPMENTS....

5. MATERIALS AND EQUIPMENTS

5.1. List of Materials used with Sources

Table 5.1: List of Materials and their Suppliers

S. No.	Name of Material	Supplied by
1	5-fluorouracil	Bindu Pharmaceuticals, Hyderabad.
2	Gelatin	Lobachemie, Mumbai.
3	Sodium alginate	Bindu Pharmaceuticals, Hyderabad.
4	Ethylcellulose	Lobachemie, Mumbai.
5	Dil HCl	Richer health care, Hyderabad.
6	Chloroform	Lobachemie, Mumbai.
7	Na CMC	Lobachemie, Mumbai.

5.2. List of Equipments used with model:

Table 5.2:List of equipments with their make

S. No.	Name of the equipment	Make
1	Electronic balance	Shimadzu, Japan
2	UV-Visible spectrophotometer	Shimadzu, Japan
3	Standared coating pan	Ganson-india
4	FTIR Spectrophotometer	Shimadzu
5	DSC test apparatus	MettlerTeldo
6	Dissolution test apparatus	Vigo Scientifics, Mumbai
7	Digital pH meter	ElicoScientifics, Mumbai
8	Hot air oven	Precision scientific co., Chennai
9	Humidity chamber	Labtech, Ambala
10	Melting point test apparatus	Precision scientific co., Chennai
12	Phase contraction microscope	Nikon
13	SEM	Merlin-FE-SEM

PRE-FORMULATION STUDIES....

6. PRE-FORMULATION STUDIES

6.1. Characterization of Drug:

6.1.1. Colour and Appearance: *(Indian Pharmacopoeia, 2007)*

The sample was observed visually.

6.1.2.Melting Point: *(Indian Pharmacopoeia, 2007)*

Melting point of drug was determined by Melting point test apparatus.

6.1.3. Solubility: *(Indian Pharmacopoeia, 2007)*

Sparingly soluble in water; slightly soluble in ethanol (95 per cent); practically insoluble in chloroform and in ether.

6.1.4. Spectral Analysis of 5-fluorouracil: *(shaik.shabbeer. et al.. 2012)*

6.1.4.1. UV Spectral Analysis of 5-fluorouracil:

6.1.4.1.1. UV Spectral Analysis of 5-fluorouracil in methanol:

6.1.4.1.1.1. Determination of absorption maximum in methanol:

A stock solution of 5-fluorouracil (100µg/ml) was prepared by dissolving 10 mg of drug in methanol and final volume was made to100ml. A dilution of (10 µg/ml) was kept in cuvette. The solution was scanned in the range of wavelength 200 – 400 nm. The UV spectrum showingλmax was recorded using double beam UV-Visible spectrophotometer

6.1.4.1.1.2. Preparation of Standard Curve of 5-fluorouracil in methanol:

A stock solution of 5-fluorouracil (100 µg/ml) was prepared by dissolving 10 mg of drug in methanol and final volume was made to 100 ml. The solutions in concentration range of 2-12 µg/ml were prepared by appropriate dilutions of stock solution. The UV absorbances of these solutions were determined spectro photometrically at λmax 266nm using double beam UV-Visible spectrophotometer.

6.1.4.1.2. UV Spectral Analysis of 5-fluorouracil by using 0.1N HCl:

6.1.4.1.2.1. Determination of absorption maximum in 0.1N HCl:

A stock solution of 5-fluorouracil (100 µg/ml) was prepared by dissolving 10 mg of drug in 0.1N HCl and final volume was made to 100 ml. A dilution of 10µg/ml was kept in cuvette. The solution was scanned in the range of wavelength 200 – 400 nm. The UV spectrum showing λmax was recorded using double beam UV-Visible spectrophotometer.

6.1.4.1.2.2. Preparation of Standard Curve of 5-fluorouracil in 0.1N HCl:

A stock solution of 5-fluorouracil (100 µg/ml) was prepared by dissolving 10 mg of drug in 0.1N HCl and final volume was made to 100 ml. The solutions in concentration range of 2 -12µg/ml were prepared by appropriate dilutions of stock solution. The UV absorbances of these solutions were determined spectro photometrically at λmax266 nm using double beam UV-Visible spectrophotometer.

6.1.4.1.3. UVSpectral Analysis of 5-fluorouracil by using Phosphate buffer pH 6.8:

6.1.4.1.3.1. Determination of absorption maximum in Phosphate buffer pH 6.8:

A stock solution of 5-fluorouracil (100 µg/ml) was prepared by dissolving 10 mg of drug in Phosphate buffer pH 6.8and final volume was made to 100 ml. A dilution of 10 µg/ml was kept in cuvette. The solution was scanned in the range of wavelength 200 - 400 nm. The UV spectrum showing λmax was recorded using double beam UV-Visible spectrophotometer.

6.1.4.1.3.2. Preparation of Standard Curve of 5-fluorouracil in Phosphate buffer pH 6.8:

A stock solution of 5-fluorouracil(100 µg/ml) was prepared by dissolving 10 mg of drug in Phosphate buffer pH 6.8and final volume was made to 100 ml. The solutions in concentration range of 2 - 12 µg/ml were prepared by appropriate dilutions of stock solution. The UV absorbances of these solutions were determined spectrophotometrically at λmax266 nm using double beam UV-Visible spectrophotometer.

6.1.5. Infrared Spectrum: *(shaik.shabbeer. et al.. 2012)*

The infrared spectrum of Fluorouracil was recorded by using FTIR (Perkin elmer-Pharmaspec-1) instrument. A small quantity of sample was mixed with equal quantity of potassium bromide and placed in sample cell to record its IR spectra.

6.1.6. Loss on drying: *(Indian Pharmacopoeia, 2007)*

Loss on drying is the loss of weight expressed as percentage w/w resulting from volatile matter of any kind that can be driven off under specified condition. The test can be carried out on the well mixed sample of the substance.

$$\textbf{Loss on drying} = \frac{\textbf{Initial weight of substance} - \textbf{Final weight of substance}}{\textbf{Initial weight of substance}} \times 100$$

6.2. Drug - polymers compatability studies:

Drug polymers studies holds great importance in designing a formulation In drug formulation it is essential to evaluate the possible interactions between the active principle and the polymers, as the choice of the polymers should be performed in

relation to the drug delivery, to their compatibility with the same drug and to the stability of the final product.

6.2.1. Fourier Transform Infra-Red Spectroscopy (FTIR) Study:

(Shaik.shabbeer. et al.. 2012)

Fluorouracil powder was mixed with various polymers in the ratio of 1:1. Then, the samples were scanned with FTIR (Perkin Elmer-Pharmaspec-1)over a wave number range of 4000-400 cm^{-1}.

6.2.2. Differential Scanning Calorimetry Study (DSC):

(Shaik.shabbeer. et al.. 2012)

Fluorouracil powder was mixed with various polymers in the ratio of 1:1. The mixture of drug with polymers to maximize the like hood of obscuring an interaction. Mixture should be examined under Nitrogen to eliminate oxidative and pyrolytic effect at a standard heating rate (10^0C/minute) on DSC. Over a temperature range, this will encompass any thermal changes due to the mixture of drug with polymers. Thermograms of pure drug are used as a reference.

Appearance or disappearance of one or more peaks in thermograms of drug with polymer is considered as an indication of interaction.

FORMULATION OF MICROCAPSULES....

7. FORMULATION OF MICROCAPSULES

Table 7.1: Composition of microcapsules of 5-fluorouacil:

Formulation	5-fluorouracil	Na.alginate (1%)	Gelatine (1%)	Ethylcellulose (1%)	Dil.HCl	Chloroform	Na cmc (1%)
F1	150mg	50ml	50ml	_	Q.s	_	_
F2	150mg	100ml	50ml	_	Q.s	_	_
F3	150mg	200ml	50ml	_	Q.s	_	_
F4	150mg	300ml	50ml	_	Q.s	_	_
F5	150mg	500ml	50ml	_	Q.s	_	_
F6	150mg	_	_	50ml	_	25ml	100ml
F7	150mg	_	_	100ml	_	25ml	100ml
F8	150mg	_	_	200ml	_	25ml	100ml
F9	150mg	_	_	300ml	_	25ml	100ml

F1-F5 Coacervation Phase Seperation by change in pH.

F6-F9 Emulsion Solvent Evaporation Technique

EVALUATION OFMICROCAPSULES

....

8. EVALUATION OF MICROCAPSULES

- **Evaluation of Microcapsuless:**
  - **Organoleptic Properties of Microcapsules.**
    - Appearance.
    - Particle size.
  - **Evaluation of Microcapsules.**
    - Paricle size determination.
    - Percentage yield.
    - Drug content.
    - Entrapment efficiency.
    - Scanning electron microscopy.
    - Particle size distribution.
    - Zeta potential.
  - ***In-vitro* drug release studies.**
  - **Release drug data model fitting.**
  - **Stability Studies.**

8.1. ORGANOLEPTIC PROPERTIES OF MICROCAPSULES:

8.1.1. Appearance:

Thecapsules were visually observed for physical appearance of capsules.

8.1.2. Particle size:

Particle size distribution of microcapsules was determined by phase contraction microscopy. Few microcapsules are placed on glass slide and kept under the microscope.

EVALUATION OF MICROCAPSULES: *(shaik.shabbeer. et al.. 2012)*

8.2.1.Percentage yield:

The dried microcapsules were weighed and percentage yield of the prepared microspheres was calculated by using the following formula.

Percentage yield = (Weight of Microcapsules/Weight of Polymer + drug) X 100

8.2.2. Drug Content:

50 mg capsules were weighed and powdered and was transferred to a 100 ml volumetric flask and 15 ml pH 7.0 is added. The drug is extracted in pH 7.0 by vigorously shaking the stoppered flask for 2 hrs. Then the volume is adjusted to the mark with distilled water and the liquid is filtered. The drug content was determined by measuring the absorbance at 266 nm after appropriate dilution. The drug content was calculated using the standard calibration curve. The mean percent drug content was calculated.

8.2.3. Estimation of Entrapment efficiency:

To evaluate the amount of the drug inside the microspheres, an indirect method was used. Aliquots from the filtered solutions remaining after removal of the microspheres were assayed spectrophotometrically. The amount of drug entrapped was calculated from the difference between the total amount of drug added and the

amount of drug found in the filtered solution. About 100 mg of microspheres were completely dissolved in 500 ml of phosphate buffer solutions (pH 7.4), and stirred for 1h. Then, 2 ml of solution was filtered and the concentration of drug was determined spectrophotometrically by UV. Efficiency of drug entrapment was calculated in terms of percentage drug entrapment (PDE) as per the following formula

$$\textbf{Percentage drug entrapment efficiency} = \frac{\textbf{W initial drug} - \textbf{W free drug}}{\textbf{W initial drug}} \times 100$$

8.2.4. Loss on drying:

Loss on drying is the loss of weight expressed as percentage w/w resulting from volatile matter of any kind that can be driven off under specified condition. The test can be carried out on the well mixed sample of the substance.

$$\textbf{Loss on drying} = \frac{\textbf{Initial weight of substance} - \textbf{Final weight of substance}}{\textbf{Initial weight of substance}} \times 100$$

8.2.4. Scanning electronmicroscopy:

Morphological examination of the surface and internal structure of the dried beads was performed by using a scanning electron microscope (SEM). Microcapsules before dissolution only subjected to SEM study since, after dissolution the capsules become swollen palpable mass. Photographs were taken within the range of 50-500 magnification.

8.2. *IN-VITRO* DRUG RELEASE STUDIES:

8.2.1. Drug release studies in 0.1 N HCl: *(shaik.shabbeer. et al.. 2012)*

Drug release studies were carried out by using USP dissolution type II test apparatus. The capsules were tested for drug release for 2 hours in 0.1N HCl (750ml) as the average gastric emptying time is about 2 hours. 5ml of samples were withdrawn at the interval of 1 hour and diluted up to 10 ml with 0.1N HCl. The absorbances were measured at 266 nm. Using a double beam UV spectrophotometer to find out the amount of 5-fluorouracil released from Microcapsules.

8.3.2. Drug release studies in pH 6.8phosphate buffer:

(Shaik.shabbeer. et al.. 2012)

After drug release studies carried out in 0.1 N HCl, then 250 ml of trisodium phosphate was added to the dissolution media and the pH adjusted to 6.8. Tested for drug release for 10 hours. 5ml of samples were withdrawn at the interval of 1 hour and diluted up to 10 ml with pH 6.8 phosphate buffer. The absorbance was measured at 266 nm, using a double beam UV spectrophotometer to find out the amount of 5-fluorouracil released from Microcapsules.

Table 8.1. Parameters for *In Vitro* Drug Release

1	Apparatus	USP type II apparatus (Paddle type)
2	Temperature	37 $\pm$ 0.5° C
3	Initial Volume	900ml
4	Speed	100 rpm
5	Drawn volume	5 ml
6	Running time	2 hrs in 0.1N HCl, 10 hrs in phosphate buffer pH 6.8
7	Medium Replacement	Media refilling at 2 hrs and 5hrs

8.4. RELEASE DRUG DATA MODEL FITTING: *(shaik.shabbeer. et al.. 2012)*

The suitability of several equation that are reported in the literature to identify the mechanisms for the release of drug was tested with respect to the release data up to the first 50% drug release. The data were evaluated according to the following equations.

Higuchi model.

$$\mathbf{Mt=M_0 + K_0\, t}$$

Higuchi model.

$$\mathbf{Mt=M_0 + K_H\, t^{0.5}}$$

Korsmeyer-Higuchi model.

$$\mathbf{Mt=M_0 + K_k t^n}$$

Where Mt is the amount of the drug dissolved in time t. M_0 is the initial amount of drug. K_0 is the Higuchi release constant, K_H is the Higuchi rate constant, K_K is a release constant and n is the release exponent that characterizes the mechanism of drug release.

8.5. STABILITY STUDIES: *(shaik.shabbeer. et al.. 2012)*

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. Generally, the observation of the rate at which the product degrades under normal room temperature requires a long time. To avoid this undesirable delay, the principles of accelerated stability studies are adopted. The International Conference on Harmonization (ICH) Guidelines titled “Stability testing of New Drug Substances and Products” describes

the stability test requirements for drug registration application in the European Union, Japan and the States of America.

Stability studies were carried out at 40°C / 75% RH for the optimized formulation for 3 months. The microcapsules were stored at 40°C/75% RH as per ICH guidelines and various parameters (drug content and drug release profile) were monitored periodically for 3 months.

RESULTS & DISCUSSION....

9. RESULTS AND DISCUSSION

9.1.CHARACTERIZATION OF DRUG:

9.1.1. Colour and Appearance:

The drug (5-fluorouracil) colour is "White or off white Powder" as same as the reported reference.

9.1.2. Melting Point:

The Melting point of 5-fluorouracil was found to be 282°C. The reported melting point of 5-fluorouracil is 282°C-284°C. Hence, observed values are complies with IP.

9.1.3. Solubility Study:

The Solubility of 5-fluorouracil in different solvents is given below:

Table 9.1: Solubility of 5-fluorouracil in Different Solvents

S. No.	Solvent	µl	Inference
1	Acetone	130	Slightly soluble.
2	Cold water	80	Sparingly soluble.
3	Hot water	25	Soluble.
4	Di methyl formamide	5	Freely soluble.
5	DMSO	5	Freely soluble.
6	Methanol	100	Sparingly soluble.
7	0.1N HCl	125	Slightly soluble.
8	pH 6.8	30	Soluble.

9.1.4. SPECTROSCOPIC STUDIES:

9.1.4.1. UV Spectroscopy:

9.1.4.1.1. Determination of λmax and Preparation of Calibration Curve of 5-fluorouracil by using water:

UV absorption spectrum of 5-fluorouracil in water shows λmax at 266 nm. Absorbance obtained for various concentrations of 5-fluorouracil in water are given in Table 16. The graph of absorbance concentration for 5-fluorouracilwas found to be linear in the concentration range of 0– 12µg /ml. The drug obeys Beer- Lambert's law in the range of 0 – 12µg /ml.

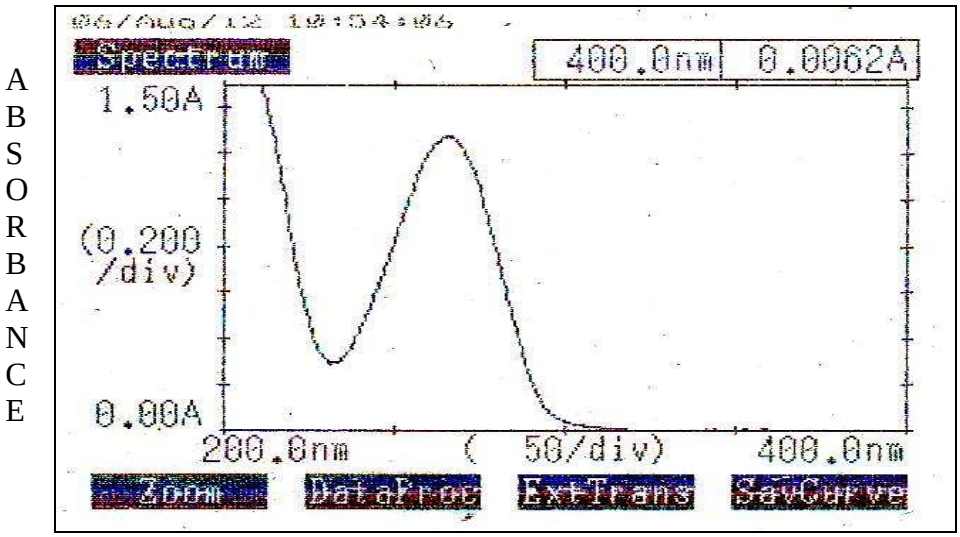


Fig. 9.1: Absorption maximum of 5-fluorouracil in water

Table 9.2: Concentration and Absorbance data for Calibration Curve of 5-fluorouracil in methanol

S. No.	Concentrations(µg/ml)	Absorbance at 266nm
1	Blank	0
1	2	0.0031
2	4	0.0062
3	6	0.0089
4	8	0.0124
5	10	0.0155
6	12	0.0186

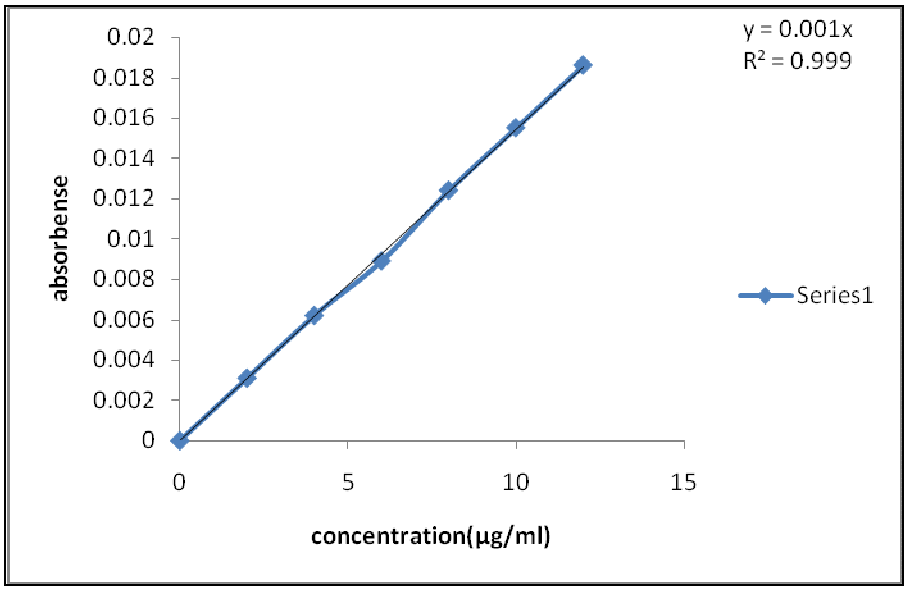


Fig. 9.2: Calibration Curve of 5-fluorouracil in water

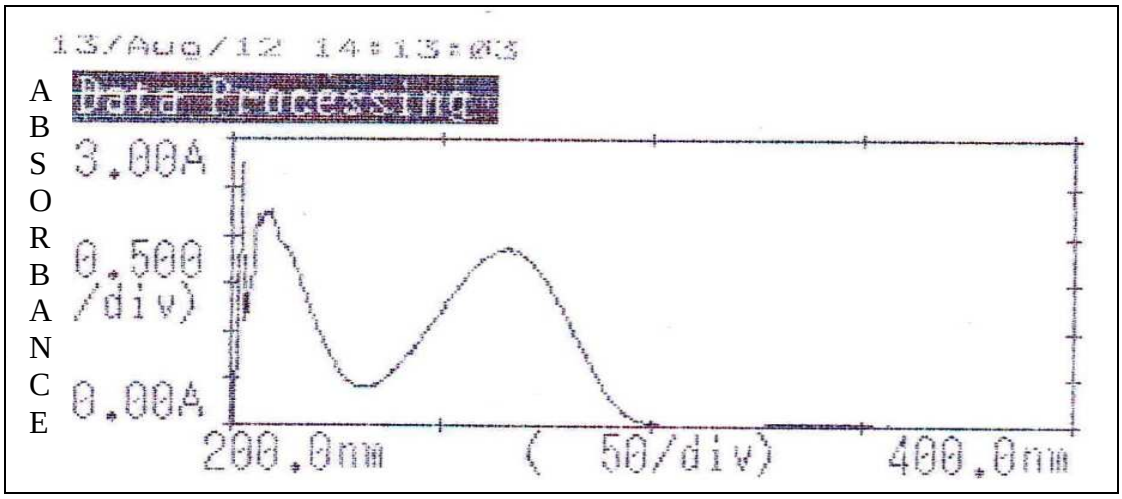
The values of Correlation coefficient (R), Slope, Intercept obtained from the calibration curve are given in the Table 9.3.

Table 9.3: Data forCalibration Curve parameters of 5-fluorouracilin methanol

S. No.	Parameters	Values
1	Slope	0.00155
2	Intercept	0.0219
3	Correlation coefficient (R)	0.995

9.1.4.1.2. Determination of λmax and Preparation of Calibration Curve of 5-fluorouracil by using 0.1N HCl

UV absorption spectrum of 5-fluorouracil in 0.1N HCl shows λmax at 266 nm. Absorbance obtained for various concentrations of 5-fluorouracilin 0.1N HCl are given in Table 18. The graph of absorbance versus concentration for 5-fluorouracil was found to be linear in the concentration range of 0 – 12µg /ml. The drug obeys Beer- Lambert's law in the range of 0– 12µg /ml.



WAVELENGTH (nm)

Fig. 9.3: Absorption maximum of 5-fluorouracil in 0.1N HCl

Table 9.4: Concentration and Absorbance data for Calibration Curve of 5-fluorouracil in 0.1N HCl

S. No.	Concentrations (µg/ml)	Absorbance at 266nm
1	Blank	0
2	2	0.0537
3	4	0.1131
4	6	0.1719
5	8	0.2321
6	10	0.3009
7	12	0.3601

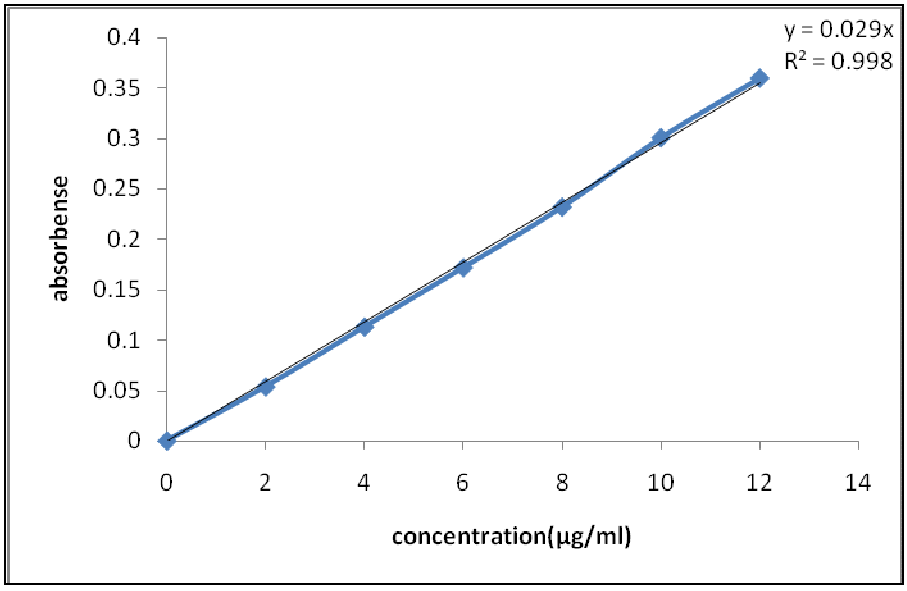


Fig. 9.4: Calibration curve of 5-fluorouracil in 0.1N HCl

The values of Correlation coefficient (R), Slope, Intercept obtained from the calibration curve are given in the Table 9.5.

Table 9.5: Data forCalibration Curve parameters of 5-fluorouracil in 0.1N HCl

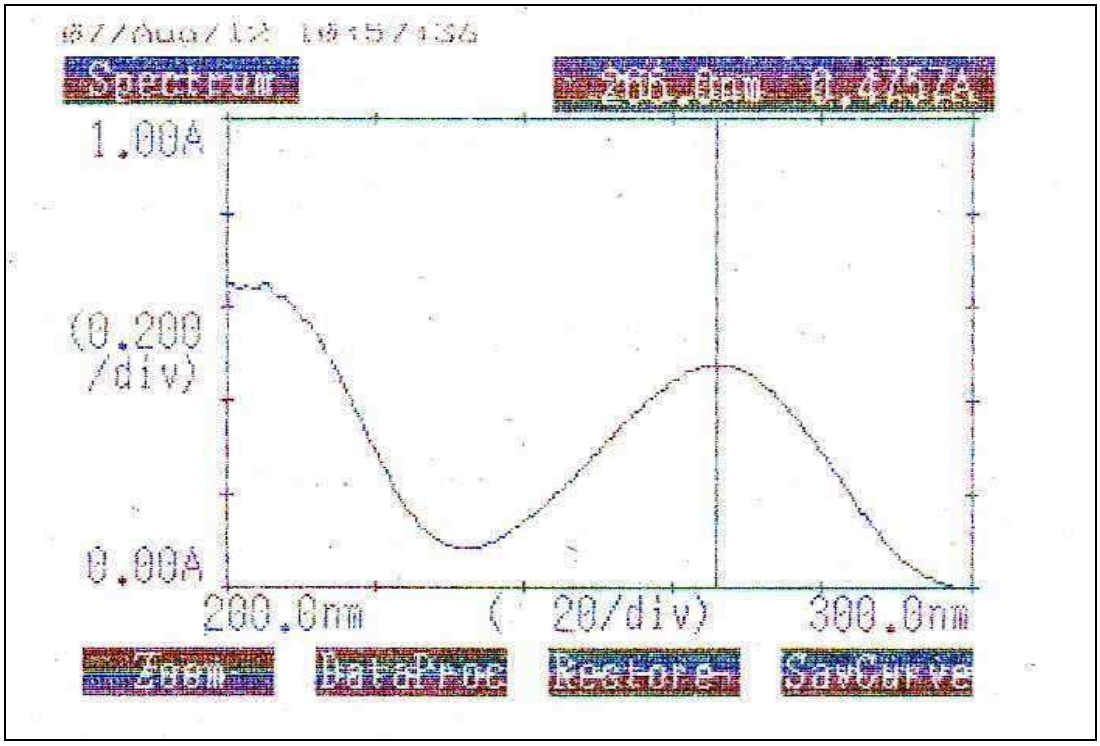
S. No.	Parameters	Values
1	Slope	0.03023
2	Intercept	0.09634
3	Correlation coefficient (R)	0.9995

9.1.4.1.3. Determination of λmax and Preparation of Calibration Curve of 5-fluorouracil by using Phosphate buffer pH 6.8:

UV absorption spectrum of 5-fluorouracil in pH6.8 phosphate buffer shows λmax at 266nm. Absorbance obtained for various concentrations of 5-fluorouracil in Phosphate buffer pH 6.8 are given in Table 20. The graph of absorbance versus concentration for 5-fluorouracil was found to be linear in the concentration range of 2 – 12µg /ml. The drug obeys Beer- Lambert's law in the range of 2 – 12µg /ml.

9.1.4.1.4. Determination of λmax and Preparation of Calibration Curve of 5-fluorouracil by using Phosphate buffer pH 6.8:

UV absorption spectrum of 5-fluorouracil in pH7.4 phosphate buffer shows λmax at 266nm. Absorbance obtained for various concentrations of 5-fluorouracil was found to be linear in the concentration range of 2 – 12µg /ml. The 5-fluorouracil absorbance in Phosphate buffer pH 6.8 is given in Table 22. The graph of absorbance concentration for drug obeys Beer- Lambert's law in the range of 2 – 12µg /ml.



ABSORBANCE

WAVELENGTH (nm)

Fig. 9.5: Absorption maximum of 5-fluorouracil in Phosphate buffer pH 6.8

Table 9.6: Concentration and Absorbance data for Calibration Curve of 5-fluorouracil in Phosphate buffer pH 6.8

S. No.	Concentration (µg/ml)	Absorbance at 266nm
1	Blank	0
2	2	0.149
3	4	0.3197
4	6	0.475
5	8	0.639
6	10	0.799
7	12	0.949

The values of Correlation coefficient (R), Slope, Intercept obtained from the calibration curve are given in the following table 9.7.

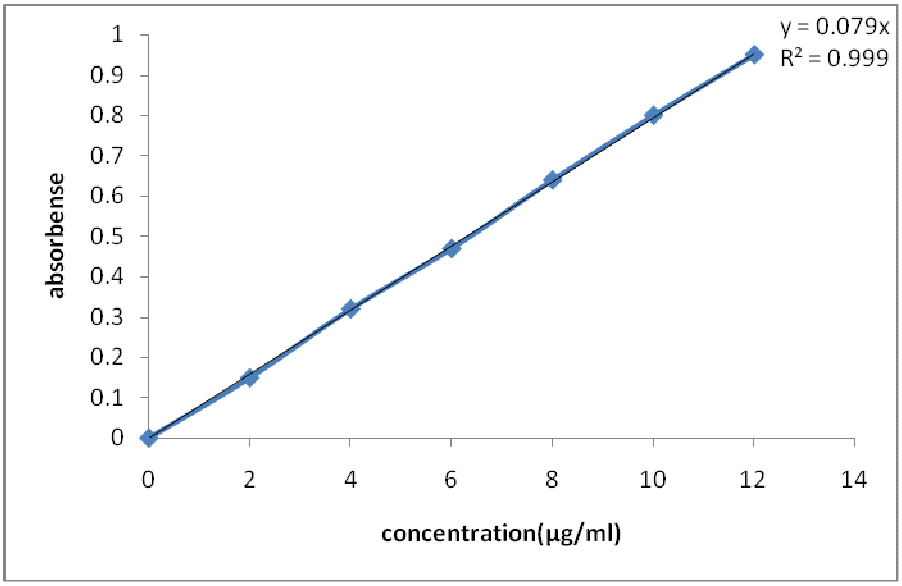


Fig. 9.6: Calibration curve of 5-fluorouracil in Phosphate buffer pH 6.8

Table 9.7: Data for Calibration Curve parameters of 5-fluorouracil in Phosphate buffer pH 6.8

S. No.	Parameters	Values
1	Slope	0.037
2	Intercept	0.0226
3	Correlation coefficient (R)	0.9996

9.1.4.2. Fourier Transform Infra-Red Spectroscopy (FTIR):

The IR spectrum of 5-fluorouracil is shown in figure 15. The Interpretation of IR frequencies are shown in Table 24.

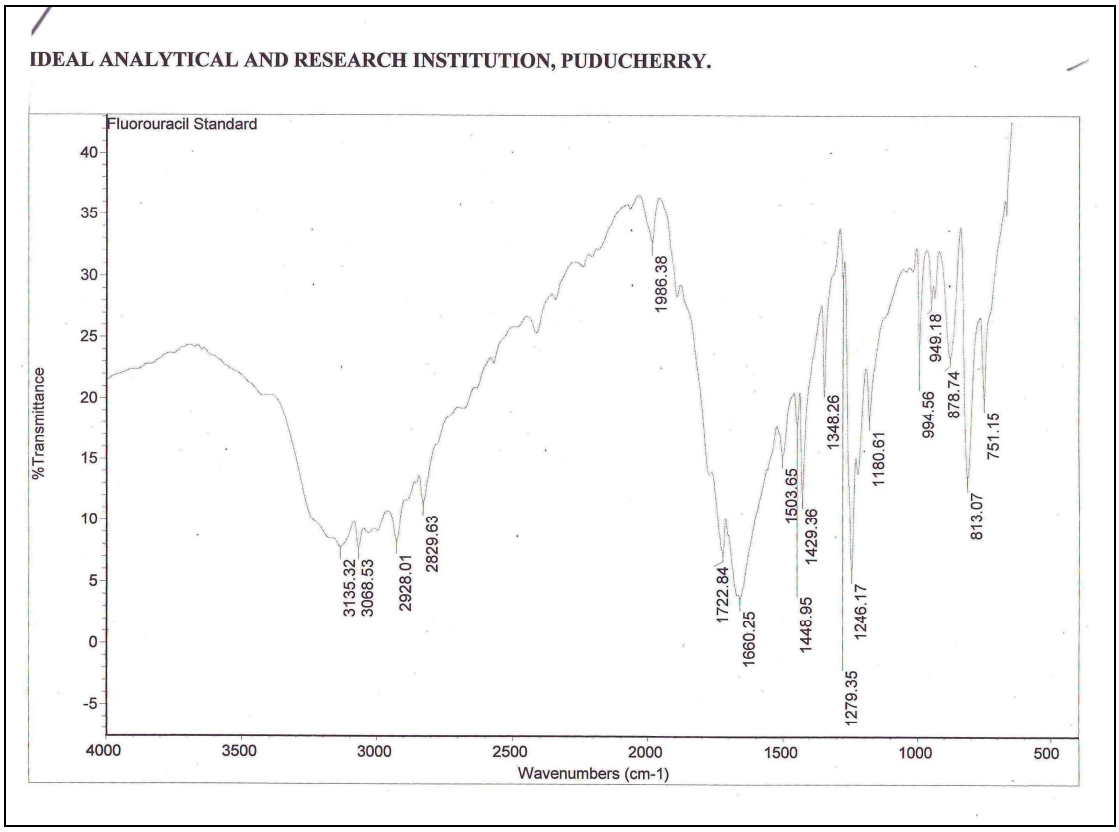


Fig. 9.7: IR Spectrum of 5-fluorouracil

Interpretation of IR Spectrum:

Table 9.8 shows the peaks observed at different wave numbers and the functional group associated with these peaks.The major peaks are identical to functional group of 5-fluorouracil. Hence, the sample was confirmed as 5-fluorouracil.

Table 9.8: Characteristic Frequencies in IR Spectrum of 5-fluorouracil

Wave nuwavenummber (cm^{-1})	Functional group
3135.32	OH Stretching
3068.53	CH Stretching
2928.01	CH_3 Asymmetric stretching
1722.84	C=O stretching
1429.36	C=O Stretching
1348.26	Symmetric CH_3 vibration
1246.17	C-O Stretching
994.56	CH Deformation
949.18	OH Deformation
751.15	CH_2 Rocking

9.1.5. Loss on drying:

The percentage loss on drying after 5 hours was found to be 0.208±0.003%.

The sample passes test for loss on drying as per the limits specified in IP.

Table 9.9: Loss on drying of 5-fluorouracil

S. No.	Percentage Loss on drying (%)	Average LOD (%)
1	0.205	0.208±0.003
2	0.206	
3	0.214	

All the values are expressed as a mean ± SD., n = 3

9.2 DRUG - POLYMERS COMPATIBILITY STUDIES

Fig. 9.8. Fourier Transform Infra-Red Spectroscopy (FTIR):

A.

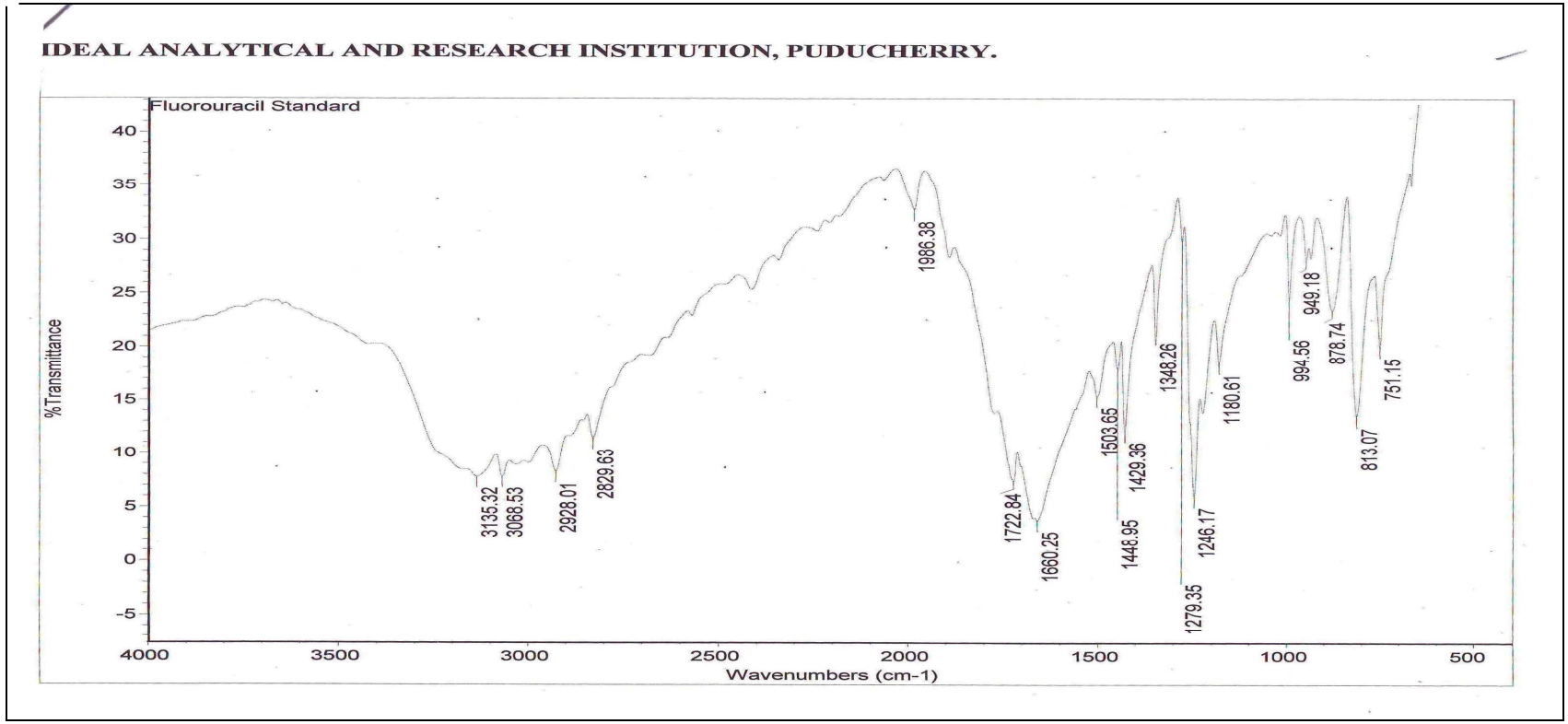


Fig.9.9. FTIR Spectrosccopy of Fluorouracil and sodium alginate

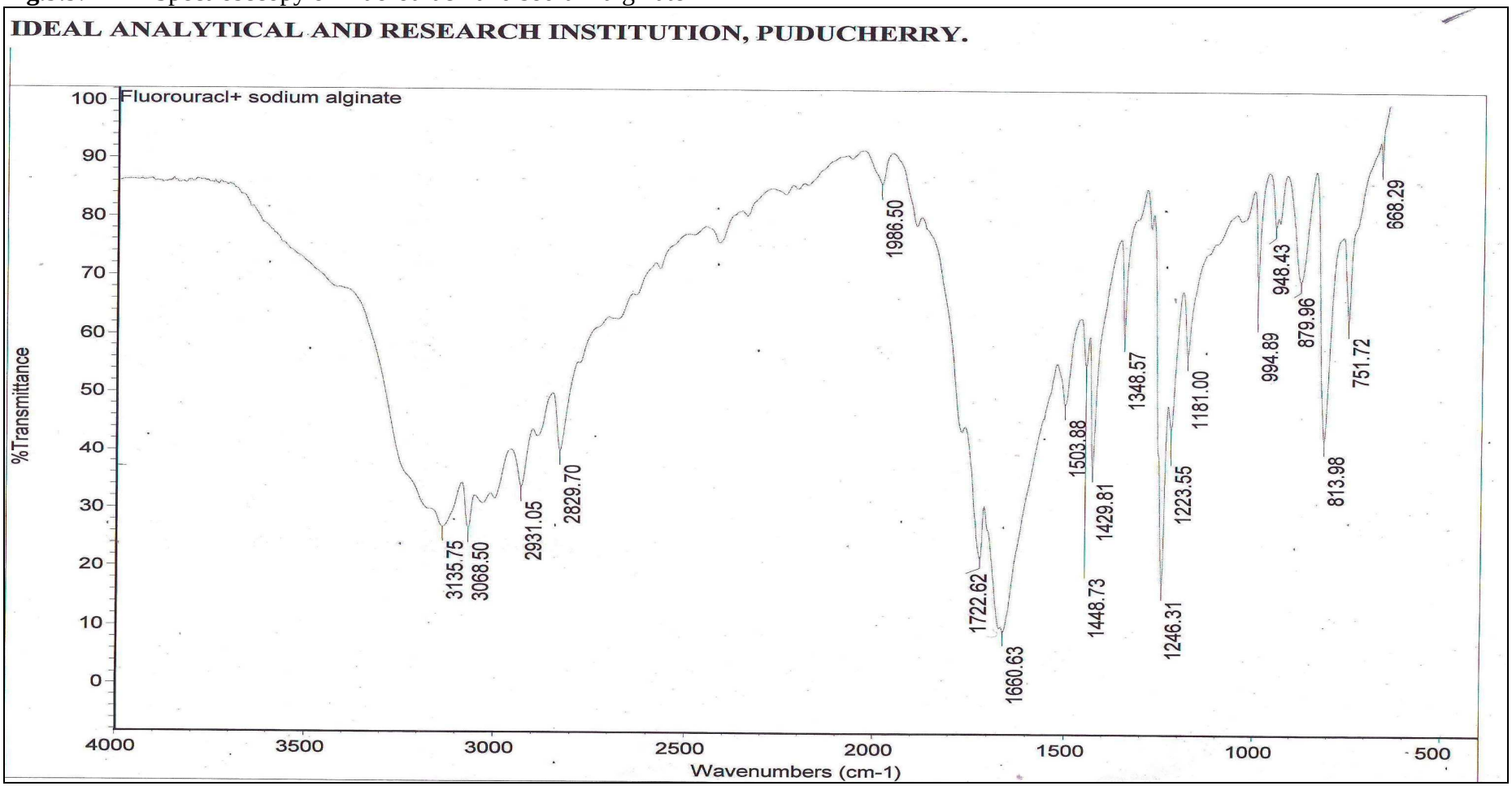


Fig. 9.10. FTIR Spectroscopy of fluorouracil and gelatin

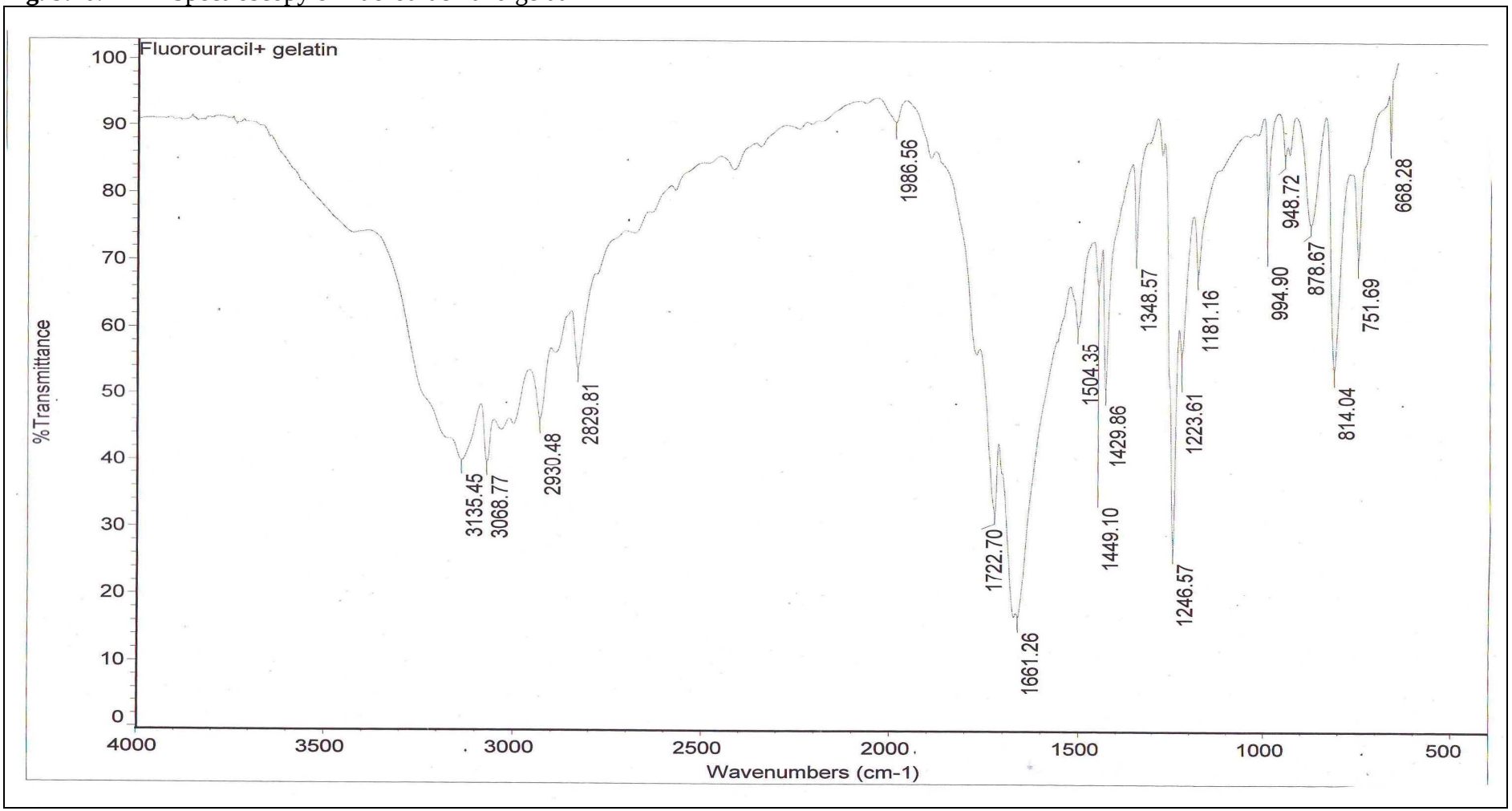


Fig.9.11. FTIR spectroscopy of fluorouracil and ethyl cellulose

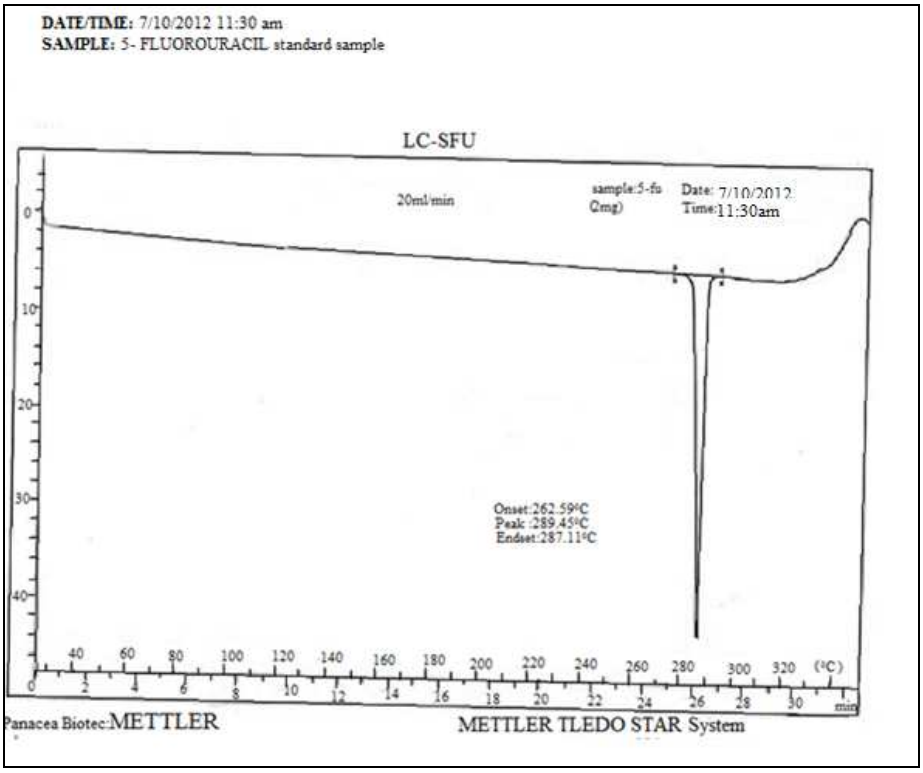


Fig.9.12. Dsc of 5-fluorouracil standard drug

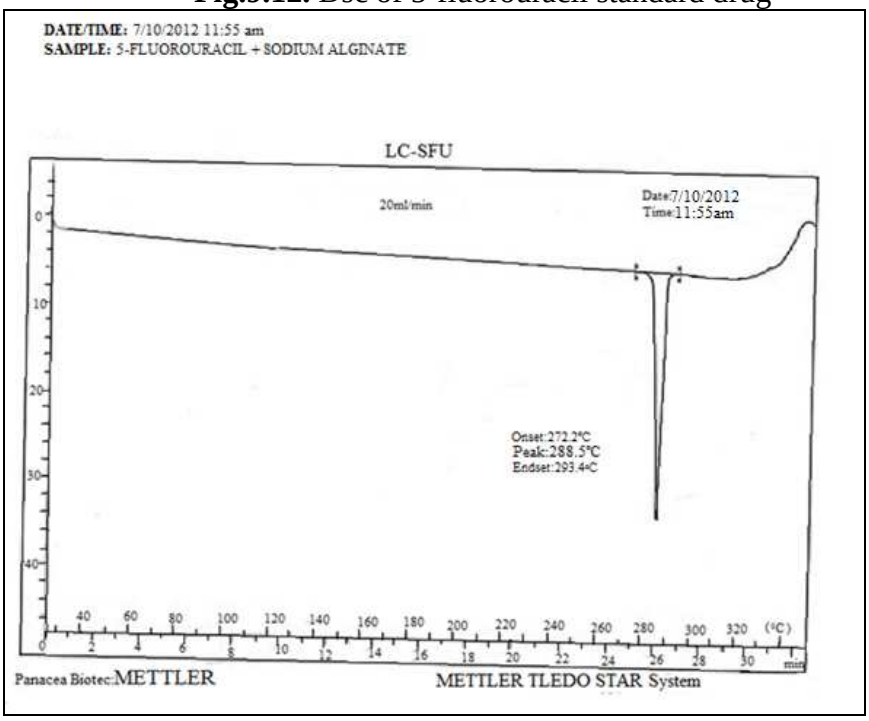


Fig.9.13. . Dsc of 5-fluorouracil + sodium alginate

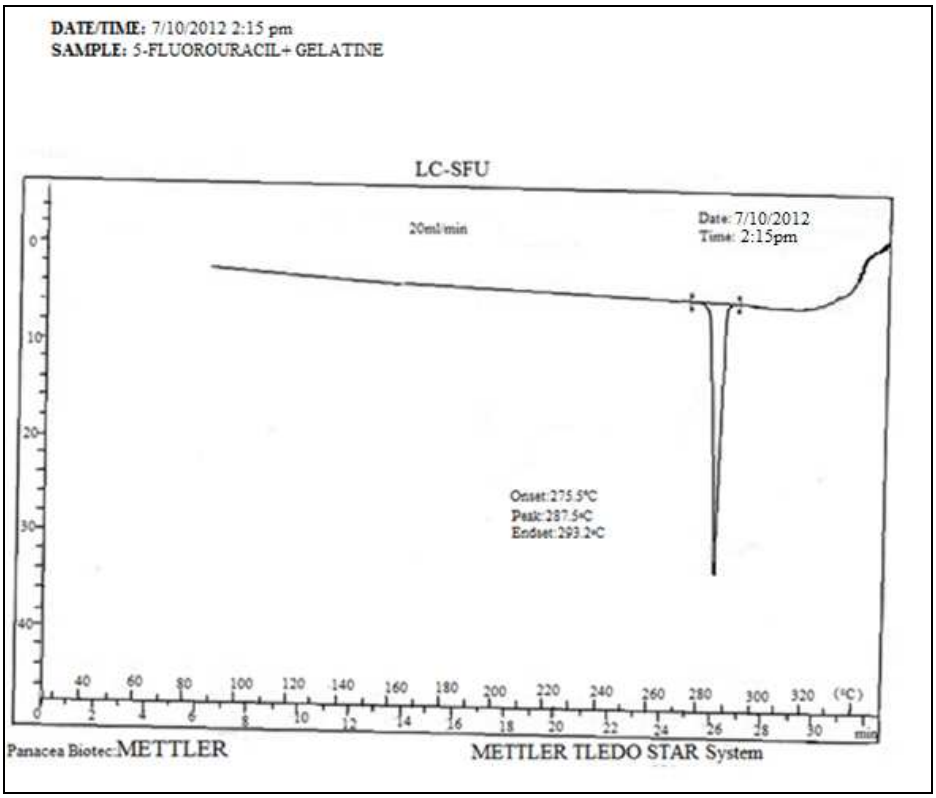


Fig.9.14. . Dsc of 5-fluorouracil + gelatine

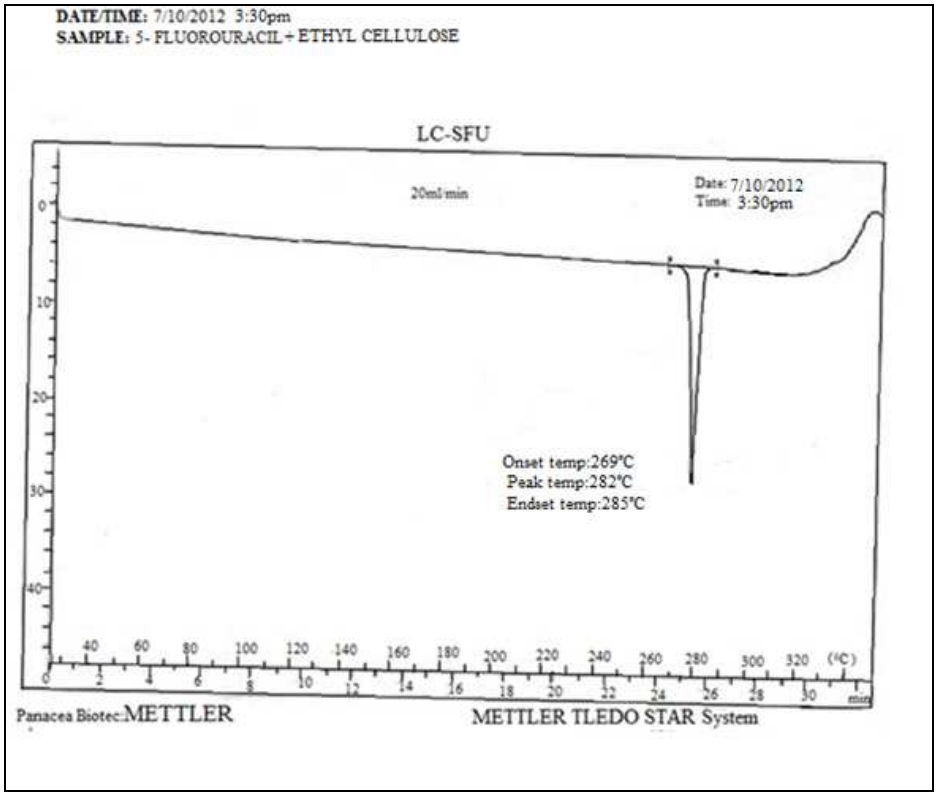


Fig.9.15. . Dsc of 5-fluorouracil+ethyl cellulose

From the above figures, it can be seen that, the major functional group peaks observed in spectra's of 5-fluorouracil with Sodium alginate, 5-fluorouracilwith gelatin and 5-fluorouracilwith ethyl cellulose remains unchanged as compared with spectra of 5-fluorouracil. So from the above IR spectra it can be observed that there is no interaction between 5-fluorouracil and Polymers used in the formulations.

9.3. ORGANOLEPTIC PROPERTIES OF 5-FLUOROURACIL MICROCAPSULES:

9.3.1. Appearance:

Table 9.10: General appearance study of microcapsules

Parameters	F1-F5	F6-F9
Composition	Gelatin and Sodim alginate	Ethyl cellulose
Shape	Spherical	Spherical
Size by visualization	Large	Small
Colour	Creamish white	More white than control
Stickiness	None	None
Odour	No	No

9.3.1.1. Appearance:

The phase contraction microscope shows the capsules being spheroid in shape and showing smooth surface of capsules.

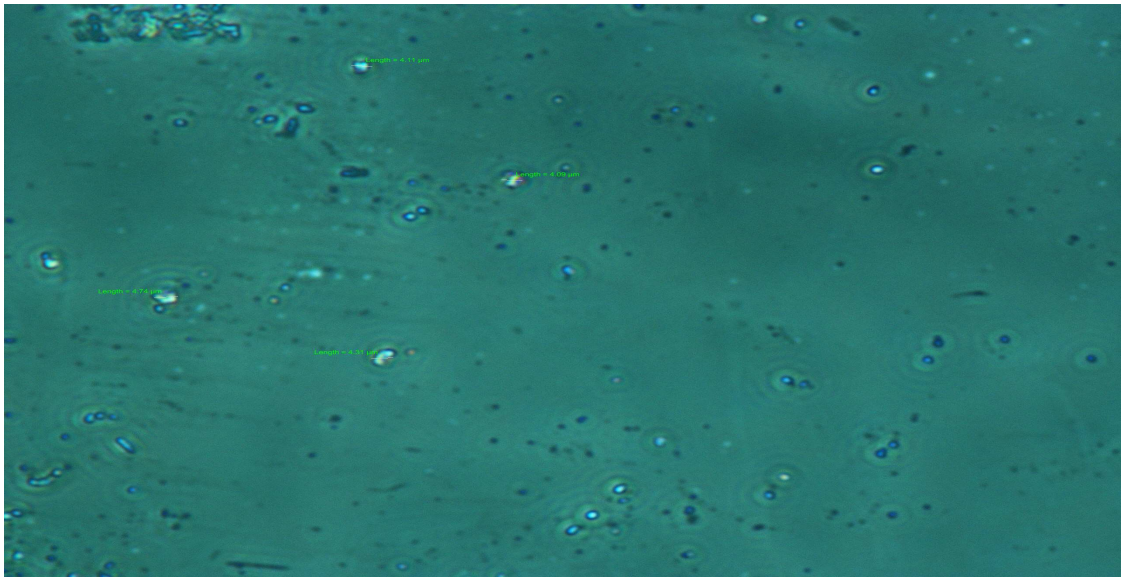


Fig.9.16. Particle size analysis by phase contraction microscopy

9.3.2.Particle size:

Table 9.11: particle size for various formulations of microcapsules

Formulations Code	Particle size (µm ± S.D)
F1	205.97±0.41
F2	207.64±0.375
F3	168.98±0.452
F4	469.72±0.271
F5	515.74±0.376
F6	14.56±0.166
F7	10.99±0.336
F8	5.60±0.150
F9	4.31±0.240

All the values are expressed as a mean ± SD., n = 3

9.3.2.1. Particle size:

The size of micro capsules found to be in the range of 4.31 µm to 515.74µm and it was observed that increase in concentration of coating polymer particle size of themicro capsules significantly increased. The average particle size is highest for F9. Theparticle size distribution is uniform and narrow.

9.4. EVALUATION OF 5-FLUOROURACIL MICROCAPSULES:

Table 9.12: Physico-Chemical Properties of microcapsules:

Formulations Code	% Yield	Drug Content* (%)	% Entrapment
F1	79.76	53.35±0.94	83.08±1.62
F2	71.42	56.81±1.31	85.12±1.21
F3	89.26	63.61±1.71	88.70±1.08
F4	65.94	36.76±1.59	88.40±1.08
F5	61.23	38.09±1.57	76.04±1.23
F6	63.84	46.15±1.50	79.42±0.41
F7	78.26	45.01±1.36	84.84±1.46
F8	79.06	57.92±1.81	81.79±1.32
F9	92.06	75.08±1.25	88.79±1.08

All the values are expressed as a mean ±SD., n = 3

9.4.1. . Percentage Yield, Drug Content and Entrapment Efficiency

The percentage yield, Drug Content and Entrapment Efficiency of Sustained release microspheres were found to increased as the polymer ratio was increased. The maximum yield of microspheres was 92.06% in Ethyl cellulose polymer, 89.26% in Gelatin and sodium alginate polymer. Better yield of microspheres was obtained from Ethyl cellulose. Drug content and Entrapment efficiency was high in Ethyl cellulose containing formulations when compared to gelatin and sodium alginate formulations. All the formulations Percentage Yield, Drug content and Entrapment efficiency data was showed in Table 9.12.

9.4.2. Loss on drying:

The value of loss on drying was found to be (0.208%±0.003) and obese the pharmacopeia limits(Less than 0.5%).

9.4.3. Scanning electron microscope (SEM):

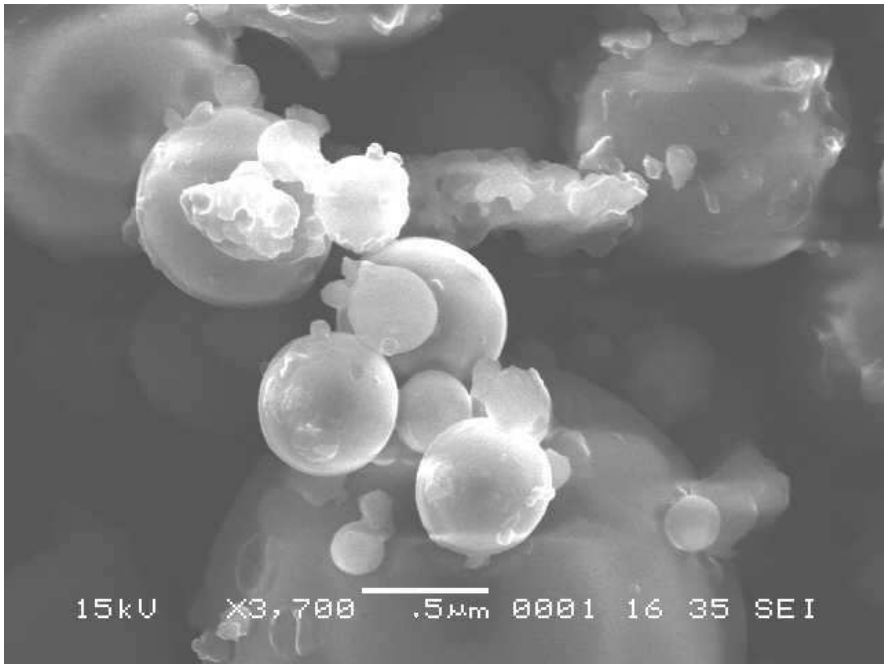


Fig.9.17.SEM image of microcapsule

Fig.9.17: Scanning electron microscopy of 5-fluorouracil loaded microcapsule

The scanning electron microscope shows the capsules being spheroid in shape. Surface depression was noted at the point of contact on the drying paper. On comparison of the capsules prepared from polymers in high concentrations more roughness was observed with ethyl cellulose polymers. Ethyl cellulose produces more smooth surface area as compared to others.

9.4.4 Particle size distribution:

Particle size analysis of fluorouracil loaded microcapsules was done by dynamic light scattering using a Malvern system and the mean particle size of fluorouracil microcapsules was found to be 4.31 figure shows the particle size distribution of fluorouracil loaded microcapsules. The polydispersity of prepared microcapsules was 10.3.

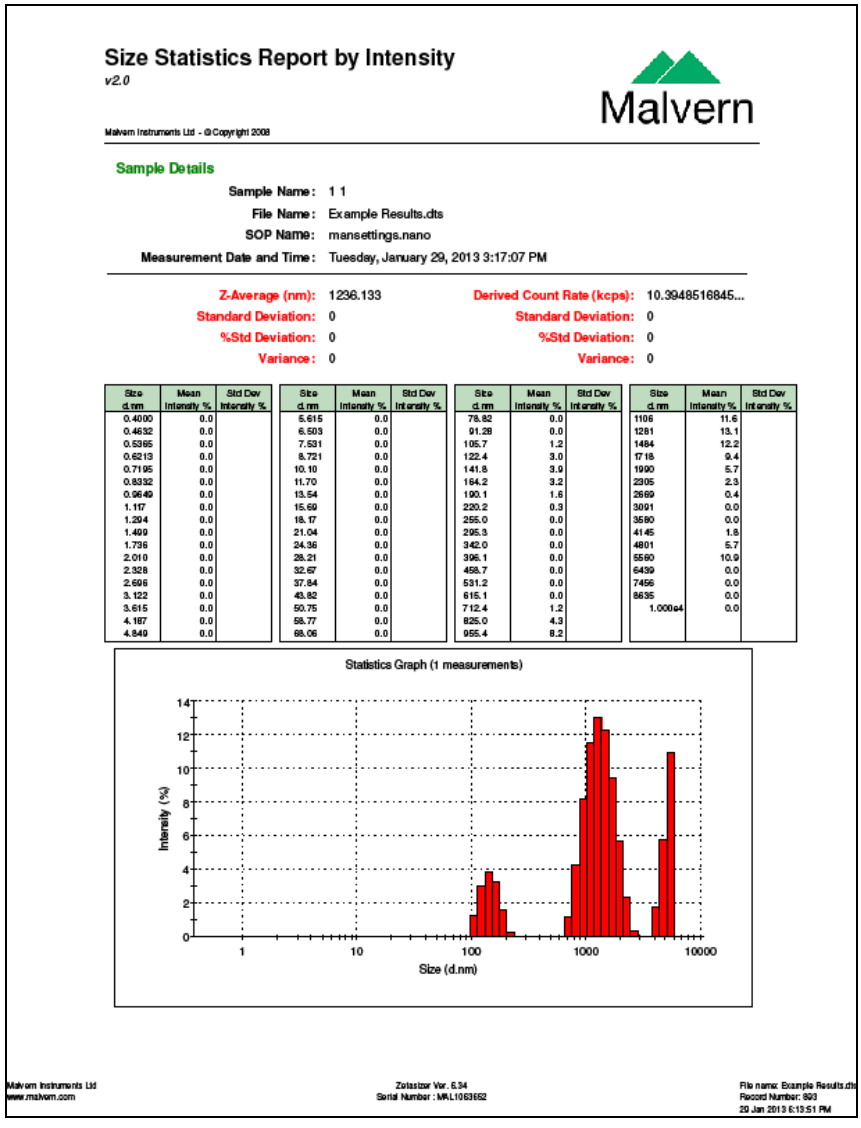


Fig.9.18. particle size distribution by using Malvern system

9.4.5.Surface charge

Surface charge analysi sof the 5-Fluorouraci lloaded microcapsules wasdone by the Malvern Zeta sizer and the zetapotential was found to be -5.60mV.Theresult of Zeta potential distribution is given in Figure 5.6

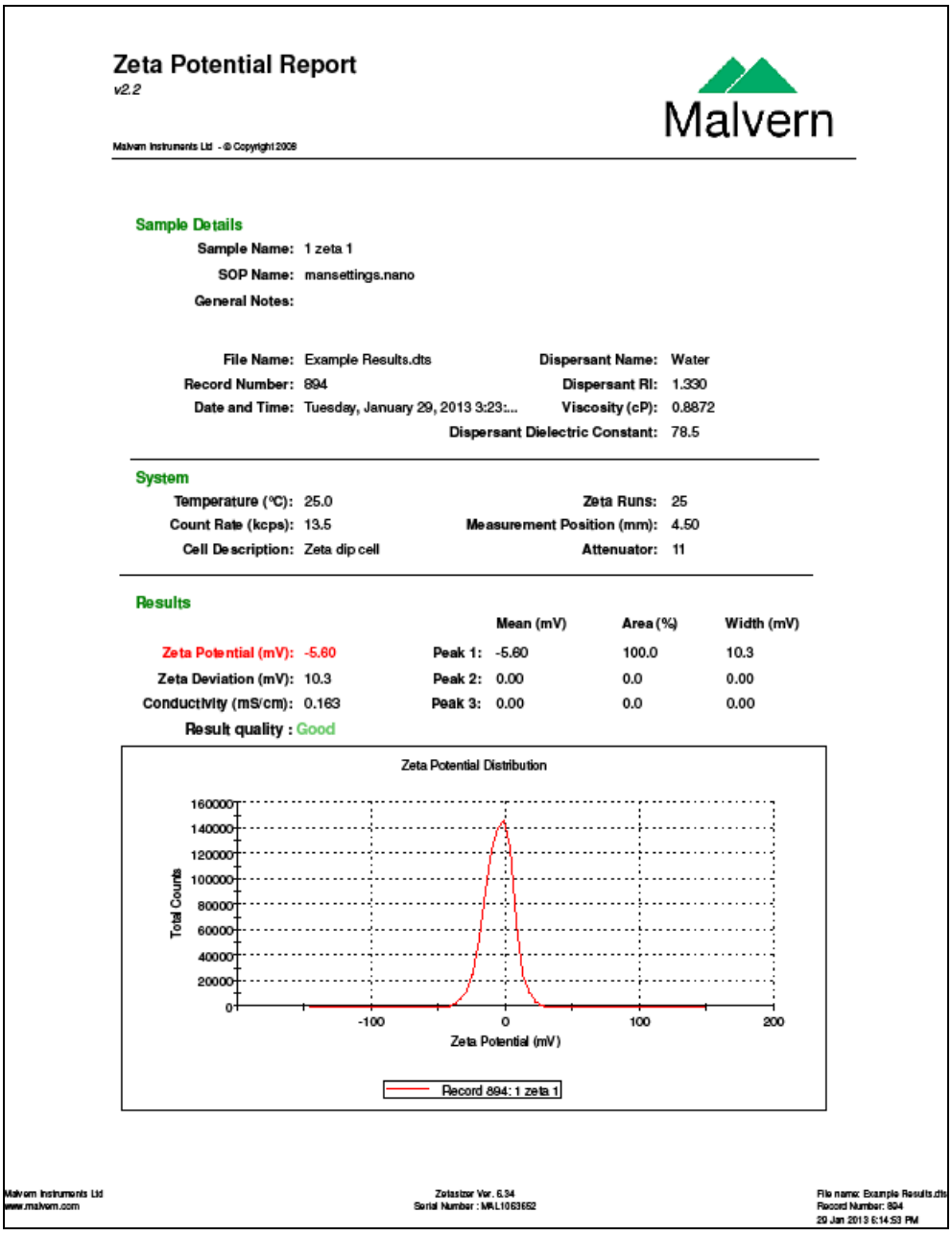


Fig.9.19.. Zeta potential determination by using Malvern system

9.5. *IN-VITRO* DRUG RELEASE STUDIES:

9.5.1. *IN-VITRO* DRUG RELEASE PROFILE OF MICROCAPSULES:

- **Drug release Profile for Formulation F1:**

Table 9.13: *In-vitro* drug release data of Formulation F1

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drugRelease (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.90±0.285	5.90	0.50	5.90
3	pH 6.8 phosphate buffer	2	6.90±0.025	6.90	0.59	12.8
4		3	10.93±0.025	10.93	1.24	16.83
5		4	17.39±0.285	17.39	2.03	23.29
6		5	20.92±0.28	20.92	2.44	26.82
7		6	51.32±0.065	51.32	4.20	57.22
8		7	71.45±0.03	71.45	4.84	77.35
9		8	74.28±0.03	74.28	4.96	80.18
10		10	75.68±0.03	75.68	5.04	81.58
11		12	76.89±0.3	76.89	5.17	82.79

All the values are expressed as a mean ±SD., n = 3

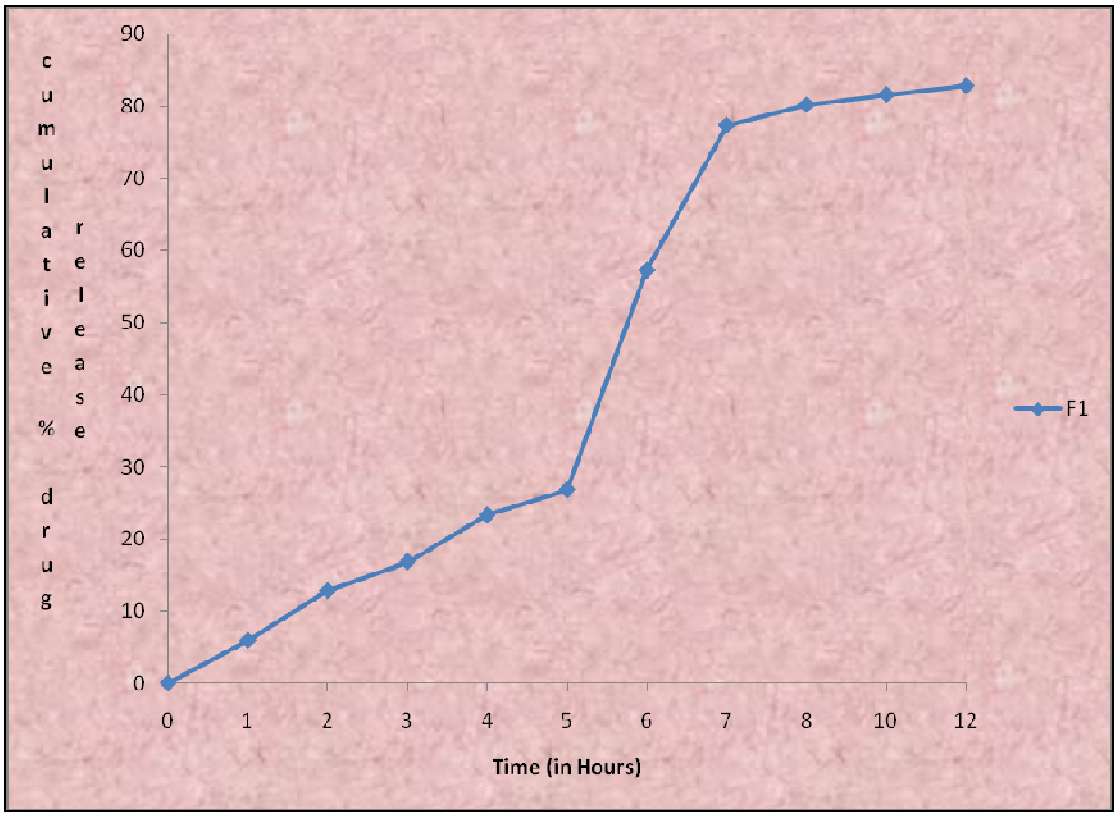


Fig.9.20. Cumulative percentage Drug release profile of F1.

❖ **Drug release Profile for Formulation F2:**

Table 9.14: *In-vitro* drug release data of Formulation F2

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drug Release (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.90±0.195	5.90	0.50	5.90
3	pH 6.8 phosphate buffer	2	6.46±0.039	6.46	0.58	12.36
4		3	10.07±0.025	10.07	1.22	15.97
5		4	17±0.03	17	2.07	22.9
6		5	20.45±0.03	20.45	2.47	26.35
7		6	50.58±0.03	50.58	4.23	56.48
8		7	71.63±0.03	71.63	4.89	77.53
9		8	75.23±0.03	75.23	5.03	81.13
10		10	76.42±0.234	76.42	5.11	82.32
11		12	76.89±0.03	76.89	5.24	81.79

All the values are expressed as a mean ±SD., n = 3

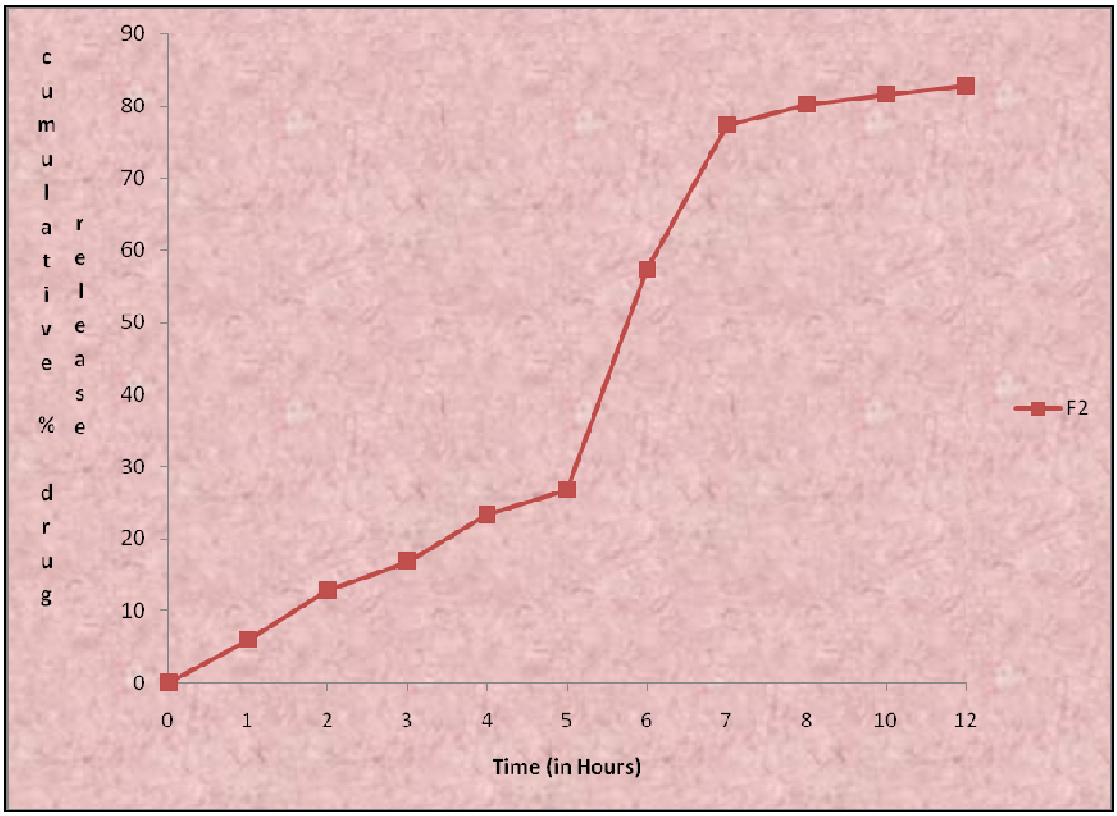


Fig.9.21. Cumulative percentage Drug release profile of F2

❖ **Drug release Profile for Formulation F3:**

Table 9.15: *In-vitro* drug release data of Formulation F3

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drug Release (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.71±0.09	5.71	0.50	5.71
3	pH 6.8 phosphate buffer	2	6.32±0.025	6.32	0.63	12.03
4		3	9.78±0.03	9.78	1.24	15.49
5		4	16.12±0.025	16.12	2.10	21.83
6		5	20.03±0.03	20.03	2.54	25.74
7		6	55.19±0.03	55.19	4.20	60.9
8		7	72.35±0.03	72.35	4.94	78.06
9		8	79.86±0.025	79.86	5.09	85.57
10		10	82.91±0.188	82.91	5.17	88.62
11		12	88.59±0.219	88.59	5.27	94.3

All the values are expressed as a mean ±SD., n = 3

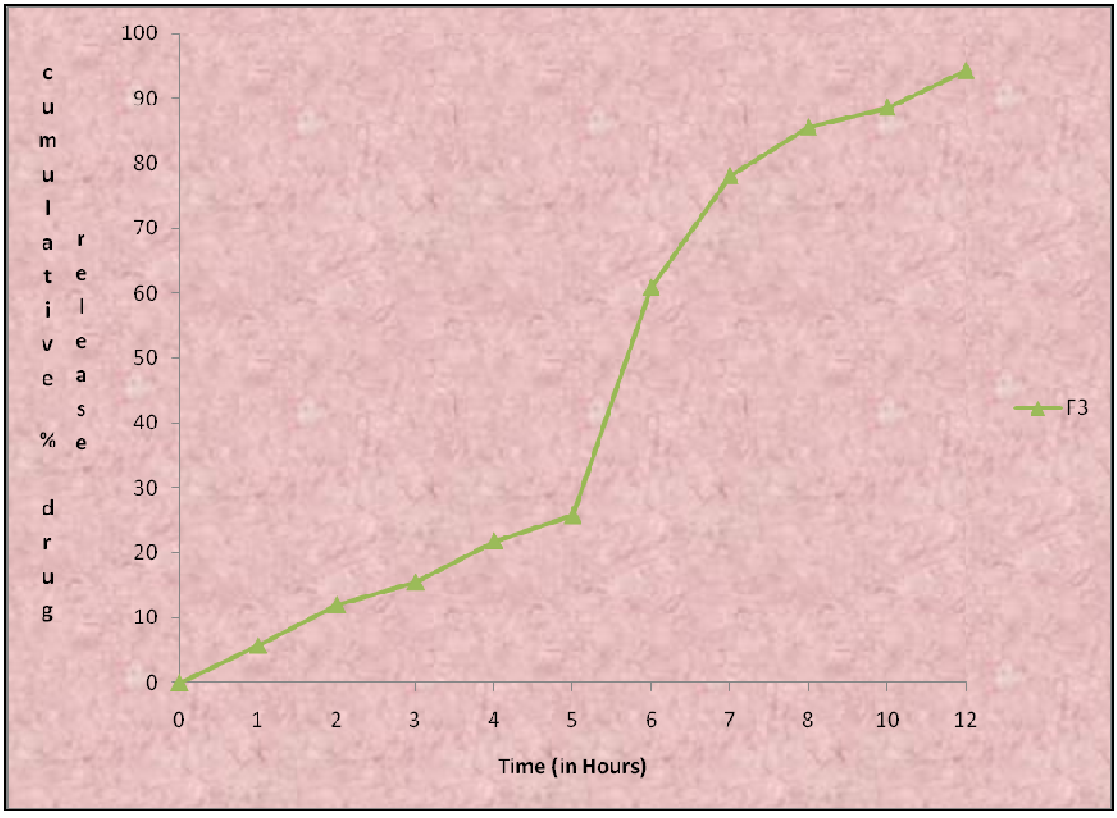


Fig.9.22. Cumulative percentage Drug release profile of F3

❖ **Drug release Profile for Formulation F4:**

Table 9.16: *In-vitro* drug release data of Formulation F4

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drug Release (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.91±0.060	5.91	0.50	5.91
3	pH 6.8 phosphate buffer	2	6.62±0.0.03	6.62	0.65	12.53
4		3	10.07±0.025	10.07	1.19	15.98
5		4	16.70±0.032	16.70	2.00	22.61
6		5	21.03±0.032	21.03	2.43	26.94
7		6	49.85±0.03	49.85	4.21	55.76
8		7	70.32±0.03	70.32	4.81	76.23
9		8	72.62±0.03	72.62	4.94	78.53
10		10	76.04±0.025	76.04	5.06	81.95
11		12	79.19±0.03	79.19	5.25	85.1

All the values are expressed as a mean ±SD., n = 3

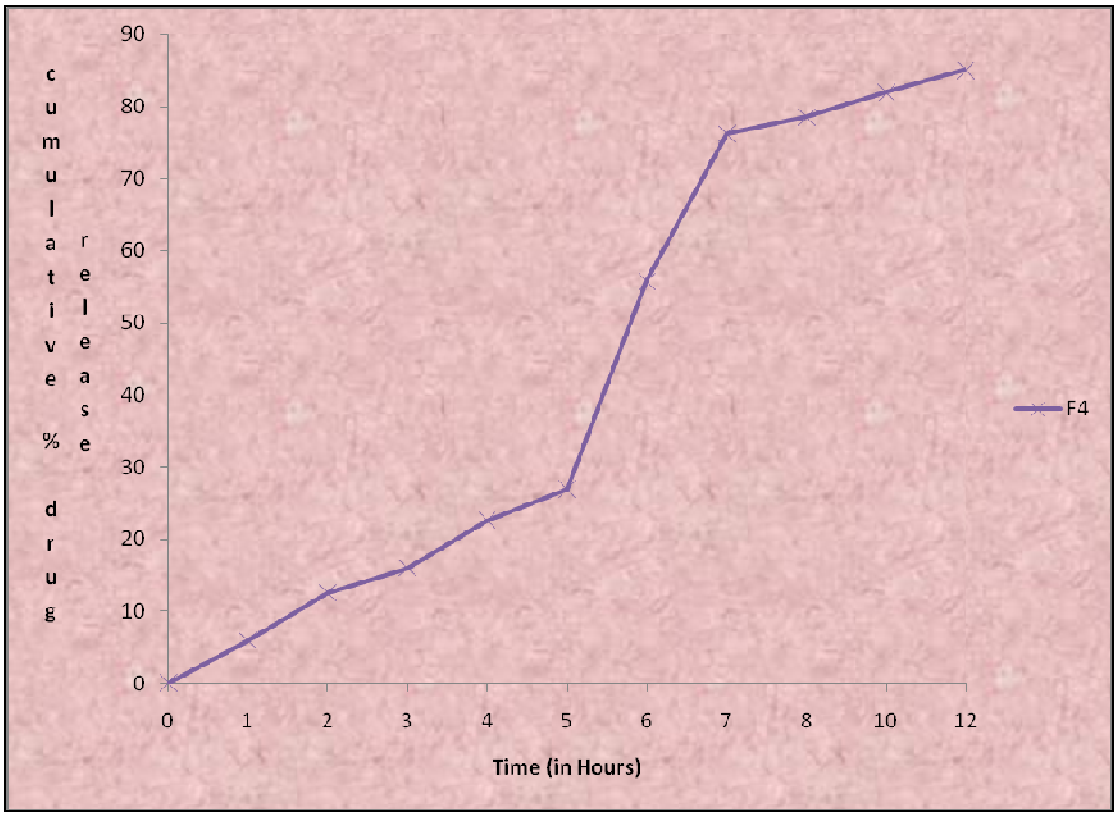


Fig.9.23. Cumulative percentage Drug release profile of F4

❖ **Drug release Profile for Formulation F5:**

Table 9.17: *In-vitro* drug release data of Formulation F5

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drug Release (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.27±0.08	5.27	0.50	5.19
3	pH 6.8 phosphate buffer	2	6.04±0.025	6.04	0.60	11.31
4		3	9.58±0.03	9.58	1.23	14.85
5		4	15.98±0.025	15.98	2.05	21.25
6		5	19.73±0.025	19.73	2.48	25
7		6	46.41±0.03	46.41	4.22	51.68
8		7	69.04±0.03	69.04	4.86	74.31
9		8	73.08±0.025	73.08	5.01	78.35
10		10	74.08±0.025	74.08	5.16	79.35
11		12	75.10±0.031	75.10	5.35	80.37

All the values are expressed as a mean ±SD., n = 3

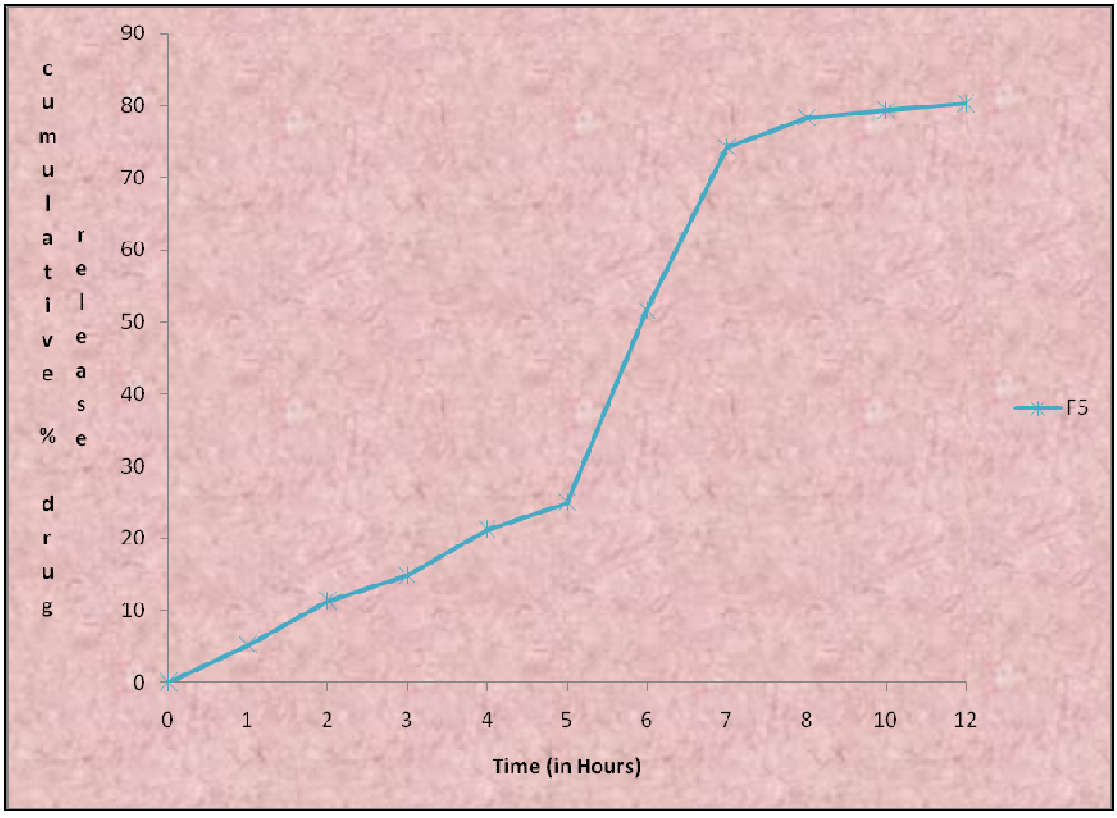


Fig.9.24. Cumulativepercentage Drug release profile of F5

❖ **Drug release Profile for Formulation F6:**

Table 9.18: *In-vitro* drug release data of Formulation F6

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drug Release (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.82±0.07	5.82	0.50	5.76
3	pH 6.8 phosphate buffer	2	6.75±0.045	6.75	0.60	12.57
4		3	12.10±0.025	12.10	1.21	17.92
5		4	19.59±0.03	19.59	2.08	25.41
6		5	24.2±0.03	24.2	2.56	30.02
7		6	56.07±0.025	56.07	4.23	61.89
8		7	74.66±0.025	74.66	4.88	80.48
9		8	76.83±0.025	76.83	4.98	82.65
10		10	78.43±0.03	78.43	5.19	84.25
11		12	80.87±0.03	80.87	5.43	86.69

All the values are expressed as a mean ±SD., n = 3

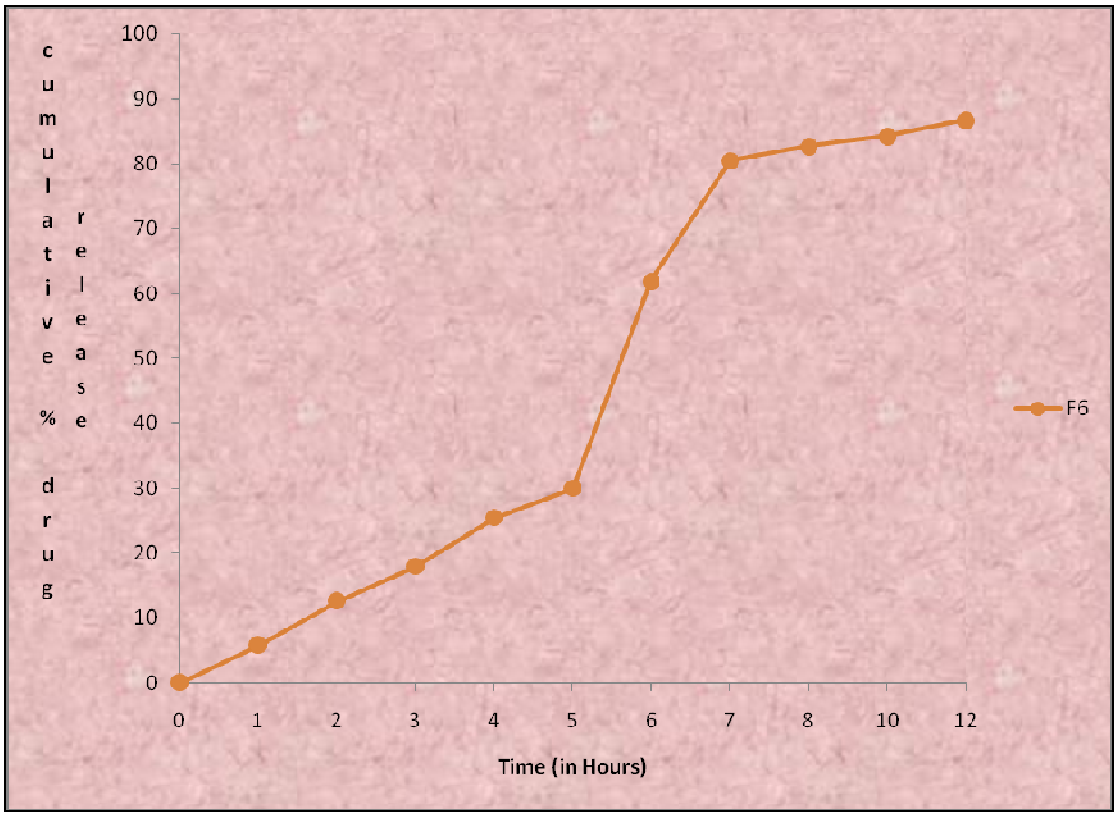


Fig.9.25. Cumulative percentage Drug release profile of F6

❖ **Drug release Profile for Formulation F7:**

Table 9.19: *In-vitro* drug release data of FormulationF7

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drug Release (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.76±0.07	5.86	0.50	5.76
3	pH 6.8 phosphate buffer	2	6.76±0.03	6.76	0.57	12.57
4		3	12.09±0.03	12.09	1.03	17.92
5		4	19.59±0.03	19.59	1.79	25.41
6		5	24.2±0.032	24.2	2.22	30.02
7		6	56.04±0.031	56.04	4.12	61.89
8		7	74.23±0.035	74.23	4.71	80.48
9		8	76.88±0.03	76.88	4.87	82.65
10		10	78.43±0.039	78.43	5.08	84.25
11		12	80.85±0.025	80.85	5.16	86.61

All the values are expressed as a mean ±SD., n = 3

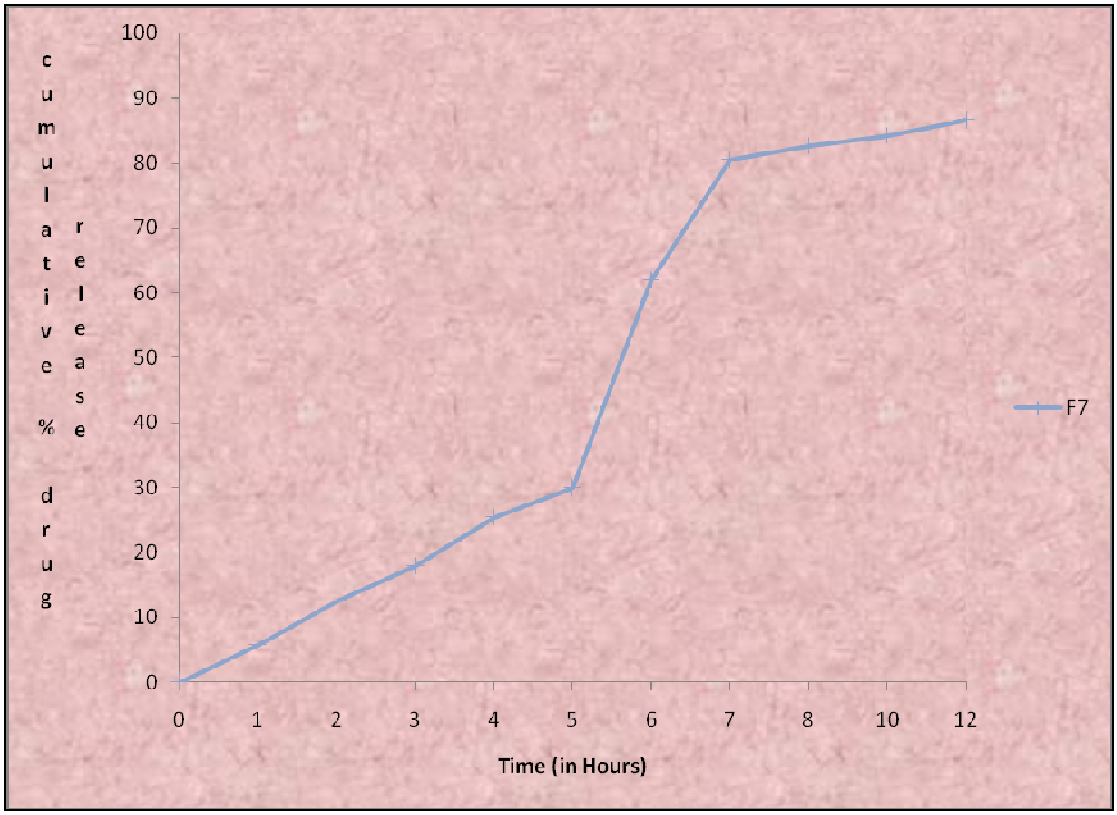


Fig.9.26. Cumulative percentage Drug release profile of F7

❖ **Drug release Profile for Formulation F8:**

Table 9.20: *In-vitro* drug release data of Formulation F8

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drug Release (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	6.36±0.102	6.36	0.50	6.36
3	pH 6.8 phosphate buffer	2	8.06±0.03	8.06	0.59	14.42
4		3	11.65±0.025	11.65	1.20	18.01
5		4	18.43±0.025	18.43	1.91	24.79
6		5	22.33±0.03	22.33	2.33	28.69
7		6	54.30±0.025	54.30	3.99	60.66
8		7	74.06±0.03	74.06	4.58	80.42
9		8	77.26±0.03	77.26	4.67	83.62
10		10	79.58±0.485	79.58	4.87	85.94
11		12	81.57±0.03	81.57	4.94	87.93

All the values are expressed as a mean ±SD., n = 3

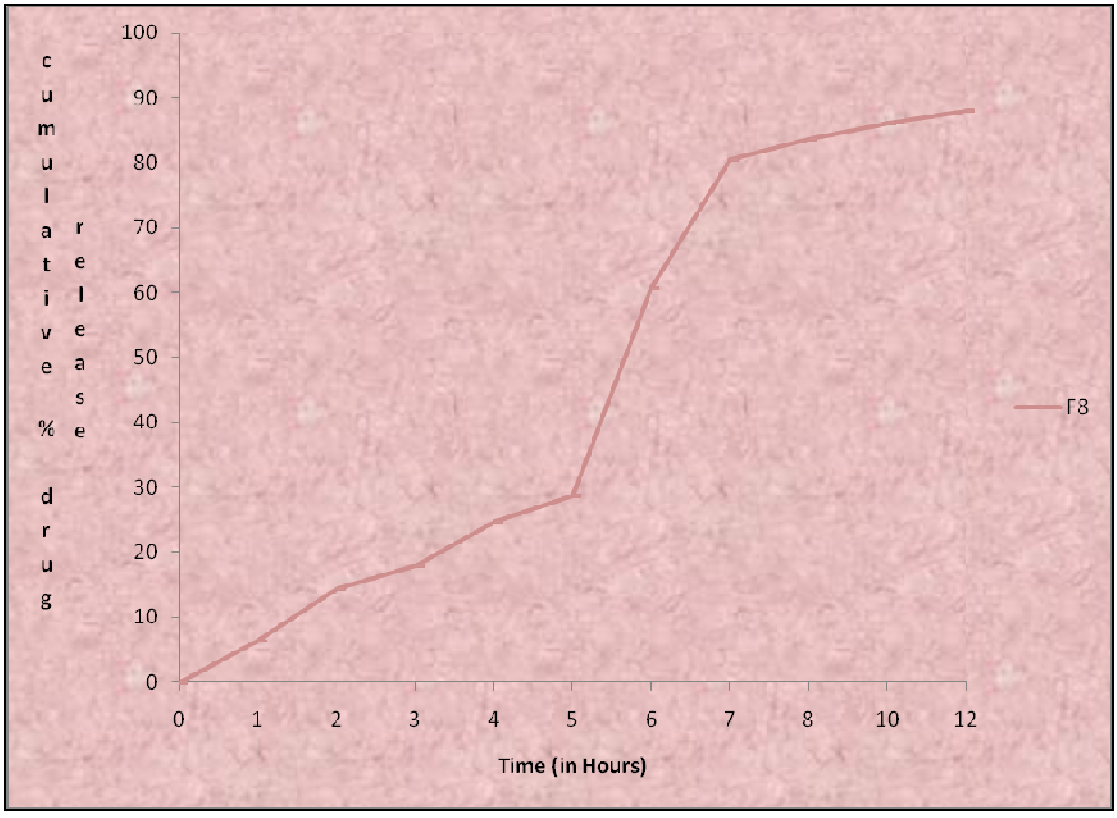


Fig.9.27. Cumulative percentage Drug release profile of F8

❖ **Drug release Profile for Formulation F9:**

Table 9.21: *In-vitro* drug release data of Formulation F9

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drug Release (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	6.05±0.02	6.05	0.50	6.05
3	pH 6.8 phosphate buffer	2	7.01±0.025	7.01	0.59	13.06
4		3	11.65±0.005	11.65	1.13	17.7
5		4	19.15±0.0251	19.15	1.88	25.2
6		5	23.77±0.002	23.77	2.31	29.82
7		6	55.47±0.005	55.47	3.98	61.52
8		7	77.21±0.0057	77.21	4.59	83.26
9		8	79.19±0.005	79.19	4.72	85.24
10		10	82.84±0.0017	82.84	4.90	88.89
11		12	88.27±0.03	88.27	5.02	94.63

All the values are expressed as a mean ±SD., n = 3

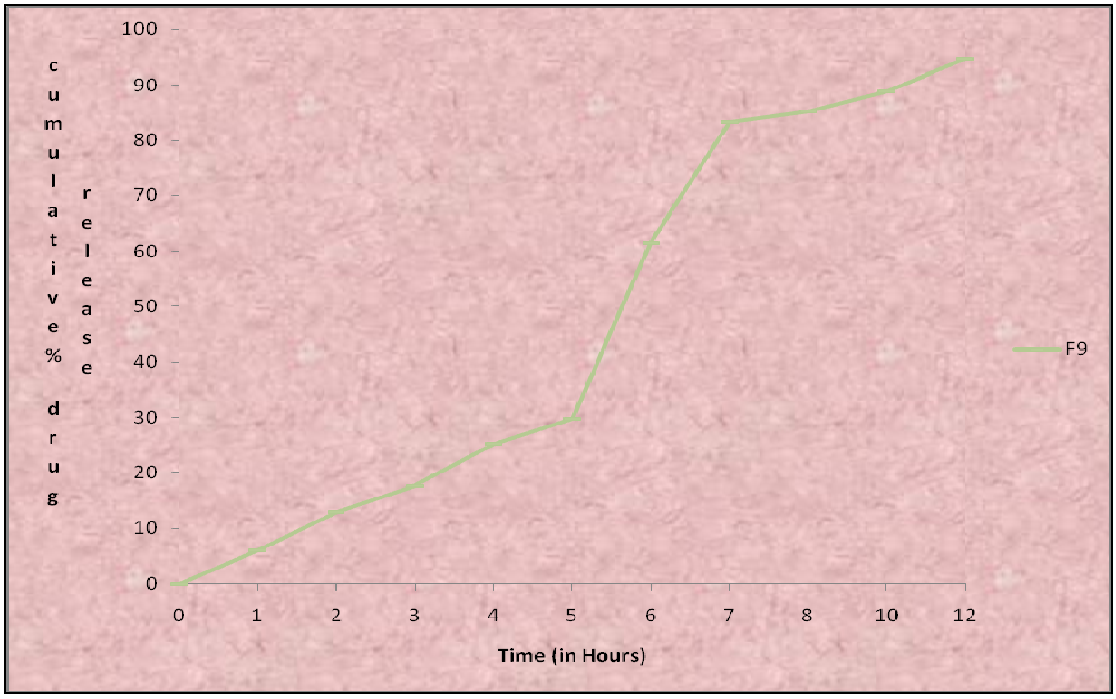


Fig.9.28. Cumulative % Drug release profile of formulation F9

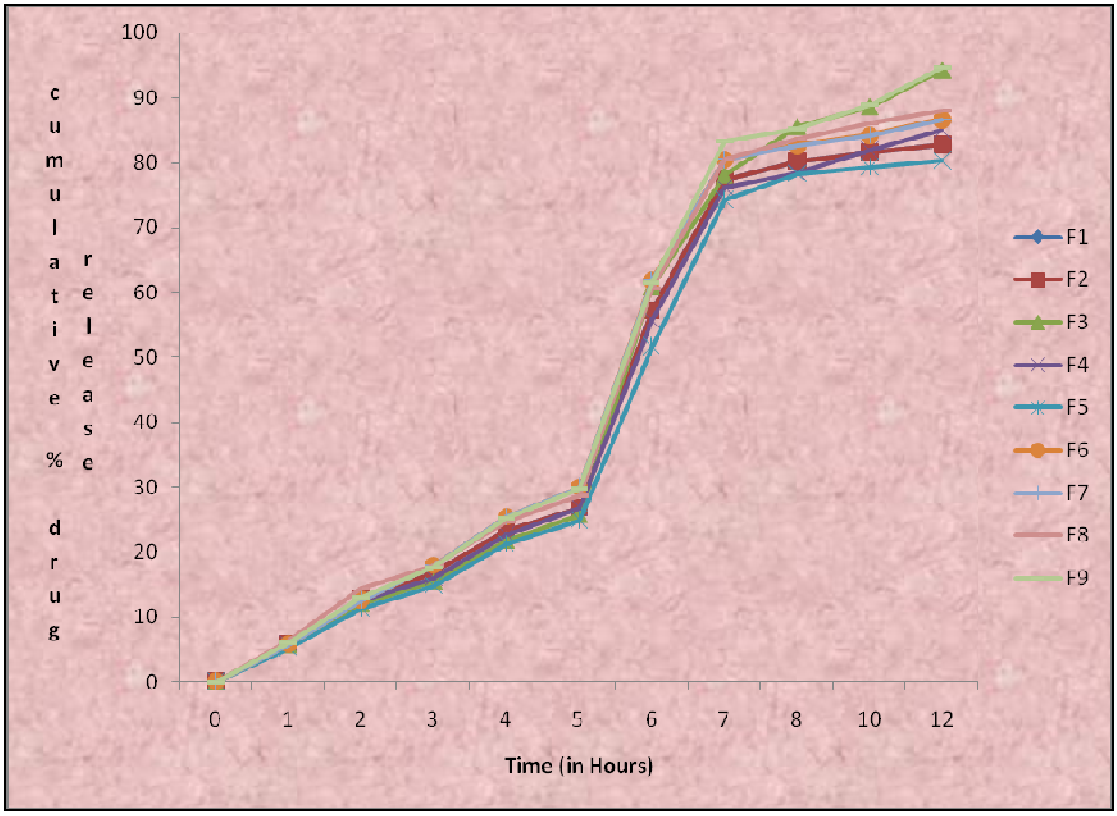


Fig.9.29. Percentage Drug release profile of formulation F1 – F9

The purpose of colon targeted drug delivery system is not only to protect the drug from being released in the physiological environment of the Stomach and Intestine but also to release the drug in the colon from the microcapsules formulation. Hence the ability of the polymers used in the formulations (F1 to F9) to retain the integrity of capsules in upper GIT were assessed by conducting drug release studies in 0.1N HCl for 2 hours and pH 6.8 phosphate buffer for 10 hours (condition mimicking mouth to the colon transit). After completing the dissolution study in 0.1 N HCl (750ml) for first two hours then, 250 ml of 0.2M trisodium phosphate was added to the dissolution media and the pH was adjusted to 6.8.samples are withdrawn after regular intervals of time to evaluate the drug release. These were analyzed spectrophotometrically at a wavelength of 266nm.

The drug release from formulation F1, F2, F3, F4 and F5was found to be 76.89%, 76.89%, 88.59%, 79.19%and 75.10% after the end of 12 hrs. This is due to lesser soluble of drug in the medium.

The drug released from formulation F6, F7, F8 and F9 containing Ethyl cellulose 80.87%, 80.85%, 81.57% and 88.27% respectively at the end of 12 hrs.

The drug released from formulation F9 containing Ethyl cellulose was found to be 88.27% at the end of 12 hrs, which is showing high percentage drug release.

9.6. RELEASE DRUG DATA MODELING:

9.6.1.Kineticsof *in-vitro* drug release:

The drug diffusion through most type of polymeric system is often best described by Fickian diffusion (diffusion exponent, n=0.5), but other process in addition to diffusion are important. There is also a relaxation of the polymer chain, which influences the drug release mechanism. This process is described as non-fickian or anomalous diffusion (n=0.5-1.0). Release from initially dry, hydrophilic glassy polymer that swell when added to water and become rubbery, show anomalous diffusion as a result of the rearrangement of macromolecular chain. The thermodynamics state of the polymer and penetrant concentration are responsible for the different type of the diffusion. A third class of diffusion is case-II diffusion (n=1), which is a special case of non- Fickian diffusion. To obtain kinetic parameter of dissolution profile, data were fitted to different kinetic models.

Table 9.22: Different Kinetic models for Formulations F1-F9

Code	Zero order		First order		Higuchi		Peppas		Best fitting model
	R^2	K_0	R^2	K_1	R^2	K	R^2	n	
F1	0.7199	0.0182	0.7203	0.0002	0.9710	0.0524	0.9554	0.3914	Higuchi
F2	0.7284	0.0182	0.7288	0.0002	0.9709	0.0524	0.9538	0.3950	Higuchi
F3	0.7815	0.7610	0.7540	0.0542	0.8934	0.0549	0.7133	0.2578	Higuchi
F4	0.8484	0.6789	0.8928	0.0549	0.9395	0.0764	0.9411	0.3533	Higuchi
F5	0.7248	0.0179	0.7252	0.0002	0.9716	0.0516	0.9558	0.3912	Higuchi
F6	0.7423	0.0849	0.8414	0.0088	0.9441	0.2727	0.9416	0.3975	Higuchi
F7	0.7336	0.0186	0.7340	0.0002	0.9744	0.0535	0.9621	0.4040	Higuchi
F8	0.7371	0.0186	0.7375	0.0002	0.9730	0.0535	0.9556	0.0643	Higuchi
F9	0.7650	0.0189	0.7653	0.0002	0.9765	0.0543	0.9593	0.3914	Higuchi

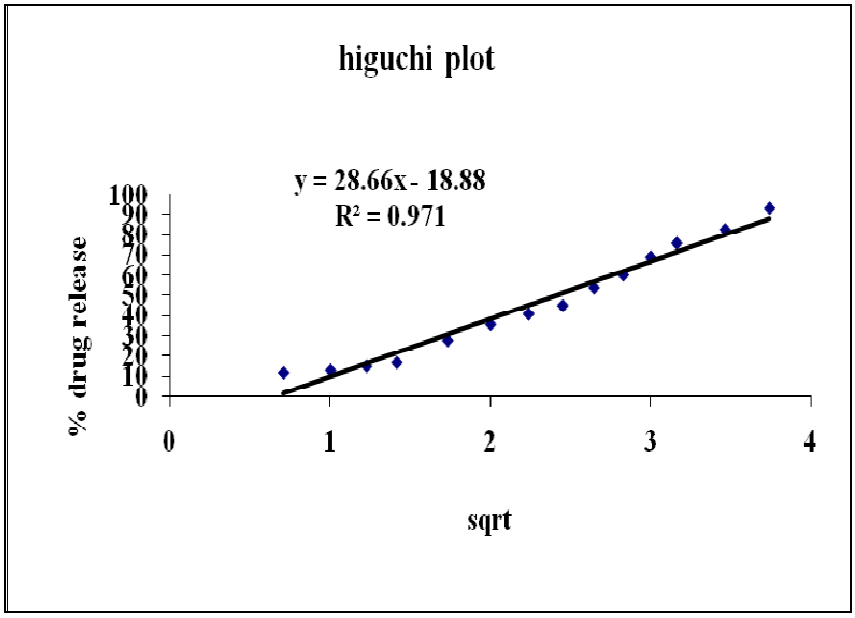


Fig. 9.30: Higuchi plot of formulation F1

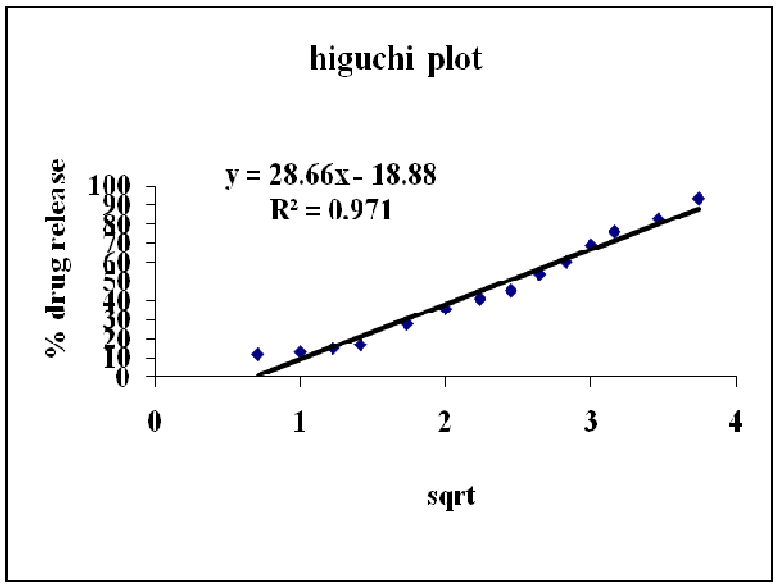


Fig. 9.31: Higuchi plot of formulation F2

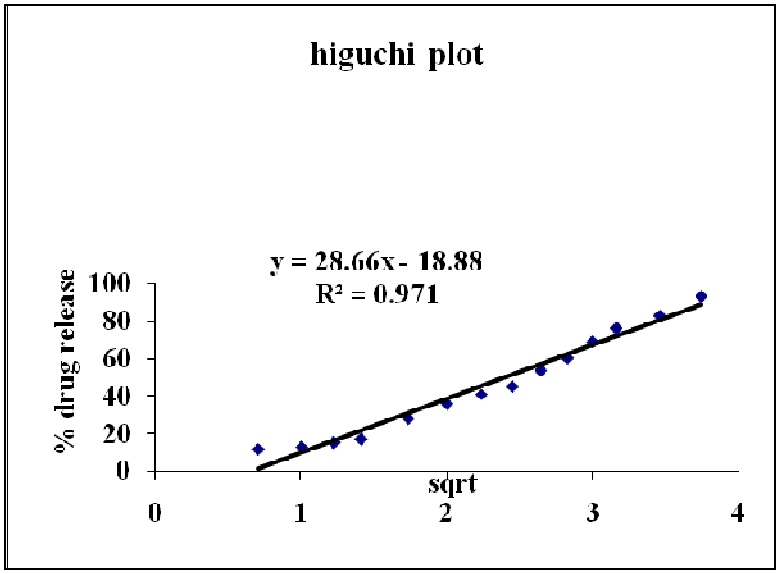


Fig. 9.32: Higuchi plot of formulation F3

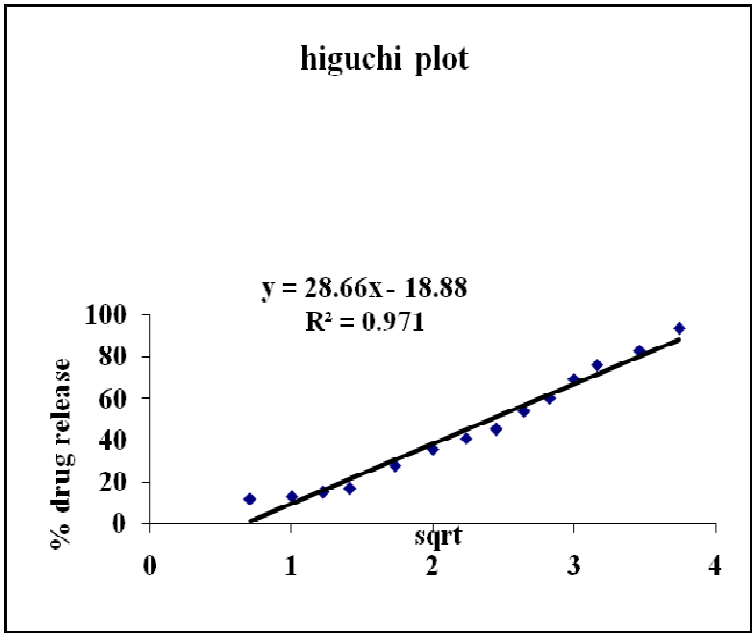


Fig. 9.33: Higuchi plot of formulation F4

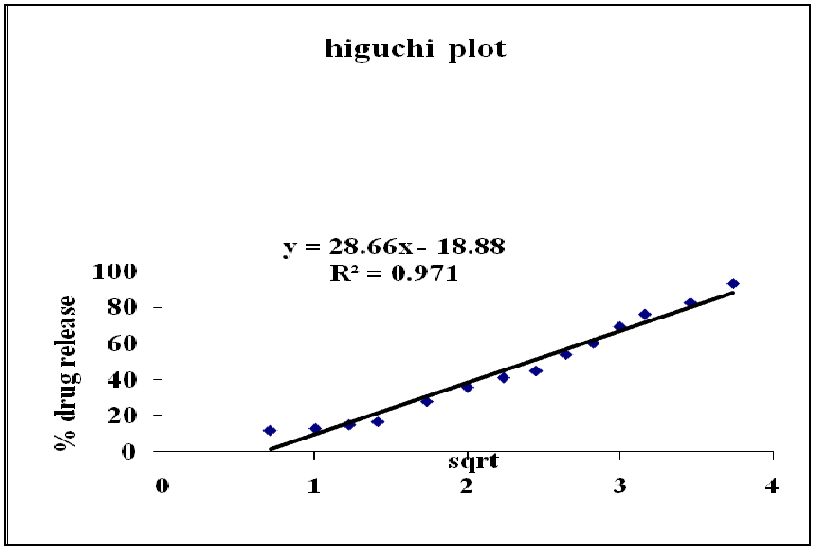


Fig. 9.34: Higuchi plot of formulation F5

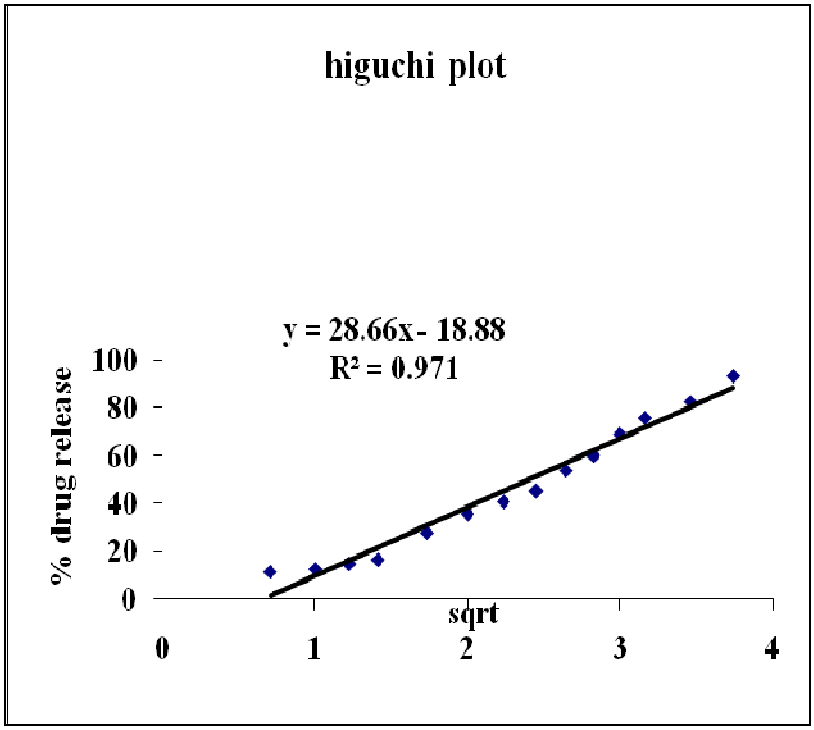


Fig. 9.35: Higuchi plot of formulation F6

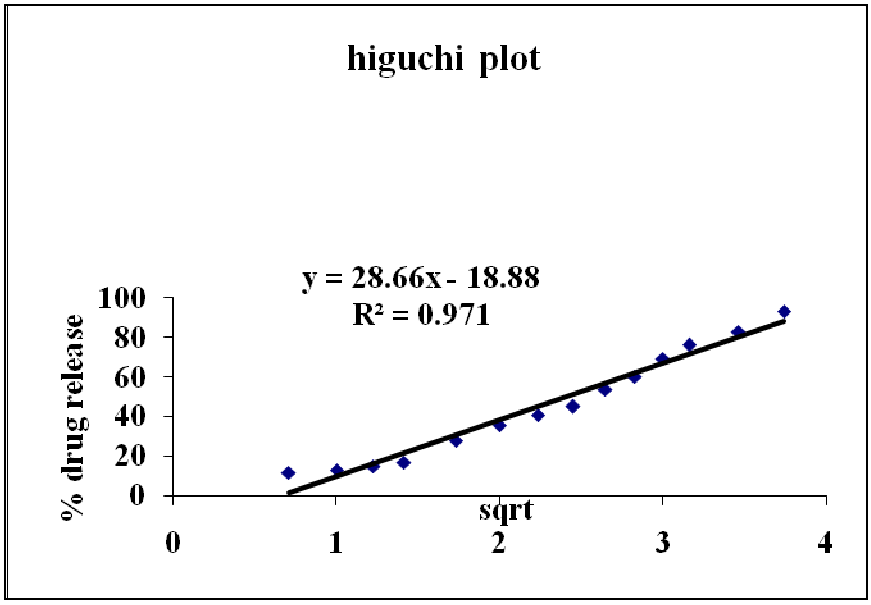


Fig. 9.36: Higuchi plot of formulation F7

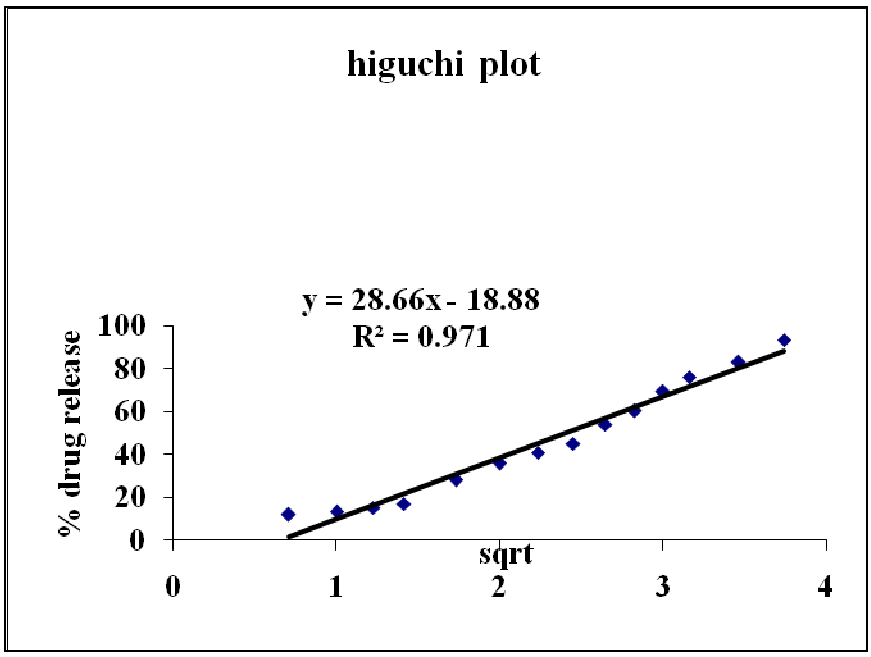


Fig. 9.37: Higuchiplot of formulation F8

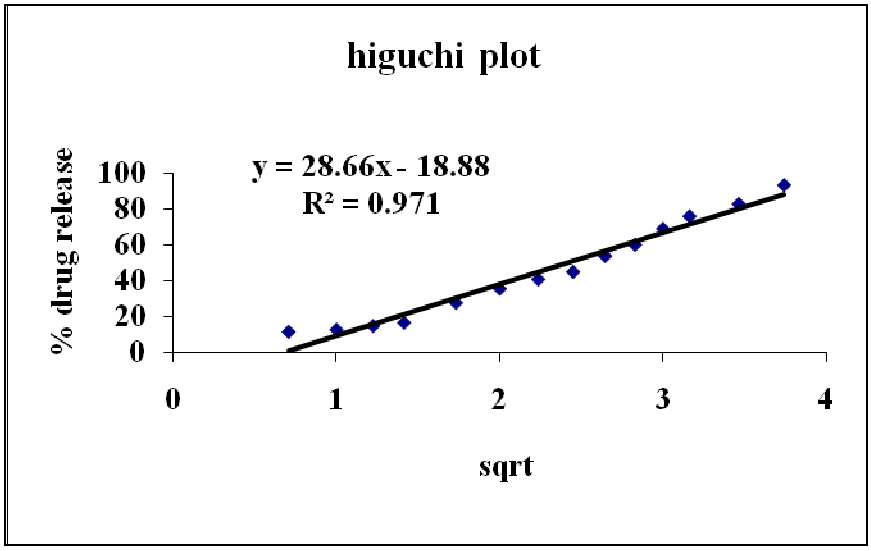


Fig. 9.38: Higuchiplot of formulation F9

For microcapsules, an "n" value near to 0.5 indicates diffusion control and an "n" value near to 1 indicates relaxation or erosion control. The intermediate value suggests that diffusion and erosion contributes to overall release mechanism.It was also observed that highest correlation was found for Higuchi log time profile (R^2> 0.99), which indicates the drug release via diffusion mechanism from all formulations.

Drug release from the formulation F9 follows the Higuchi release mechanism because its R^2 value nearer to one.

9.7. STABILITY STUDIES

From the results of the above studies it was found that formulation F9 was considered as the best formulation amongst the nine formulations. Hence formulation F9 was selected for stability studies.

9.7.1. Stability studies at the end of First month (30 days):

9.7.1.1. Content Uniformity:

The Percentage drug content of f9 micro capsules after one month of stability studies was studied. The results are within the official limits. The data is shown in Table 28.

Table 9.23: Drug content of formulation F9 at the end of 1 month of stability

S. No.	Formulation	Percentage drug content
1.	F9	74.75±0.060

All the values are expressed as a mean ±SD., n = 3

9.7.1.2.*In-vitro* drug release study:

The Cumulative Percentage Drug Release from F9 microcapsules after one month of stability was studied.The data is shown in Table 9.24.

Table 9.24: *In-vitro* drug release data of formulation F9 at the end of 1 month of stability

S. No.	Medium	Time (hours)	Drug Release (%)	Amount of drug released (mg)	MDT (hrs)	Cumulative drug Release (%)
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.71	5.71	0.50	5.71
3	pH 6.8 phosphate buffer	2	6.32	6.32	0.63	12.03
4		3	9.78	9.78	1.24	15.49
5		4	16.12	16.12	2.10	21.83
6		5	20.03	20.03	2.54	25.74
7		6	55.19	55.19	4.20	60.9
8		7	72.35	72.35	4.94	78.06
9		8	79.86	79.86	5.09	85.57
10		10	82.91	82.91	5.17	88.62
11		12	88.59	88.59	5.27	94.3

All the values are expressed as a mean ±SD., n = 3

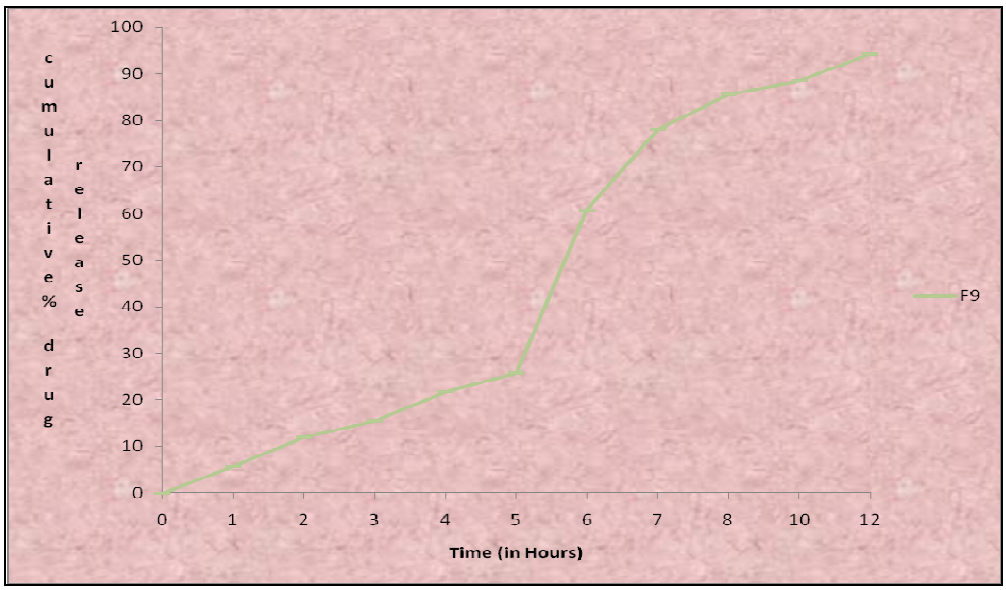


Fig.9.39. *In-vitro* drug releaseprofileof formulation F9 at the end of 1 month of stability

9.7.2. Stability studies at the end of Second month (60 days):

9.7.2.1. Drug content:

The Percentage drug content of f9 micro capsules afterTwo months of stability studies was studied. The results are within the official limits. The data is shown in Table 9.25.

Table 9.25: Drug content of formulation F9 at the end of 2 months of stability

Sl. No.	Formulation	Percentage drug content
1.	F9	74.26±0.0513

All the values are expressed as a mean ±SD., n = 3

9.7.2.2.*In-vitro* drug release study:

The Cumulative Percentage Drug Release from F9 micro capsules after Two months of stability was studied.The data is shown in Table 9.26.

Table 9.26: *In-vitro* drug releasedataof formulation F9 at the end of 2 months of stability

Sl. No.	Medium	Time (hours)	% Drug Release	Amount of drug released (mg)	MDT (hrs)	Cumulative % drug Release
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.59±0.025	0.84	0.50	5.59
3	pH 6.8 phosphate buffer	2	6.31±0.015	0.90	0.57	11.9
4		3	9.77±0.010	1.19	1.03	15.36
5		4	16.10±0.020	1.71	1.79	21.69
6		5	19.99±0.015	2.04	2.22	25.58
7		6	54.15±0.025	4.83	4.12	59.74
8		7	73.33±0.025	6.42	4.71	78.92
9		8	77.96±0.021	6.83	4.87	83.55
10		10	81.97±0.020	7.20	5.08	87.56
11		12	83.71±0.015	7.29	5.16	94.15

All the values are expressed as a mean ±SD., n = 3

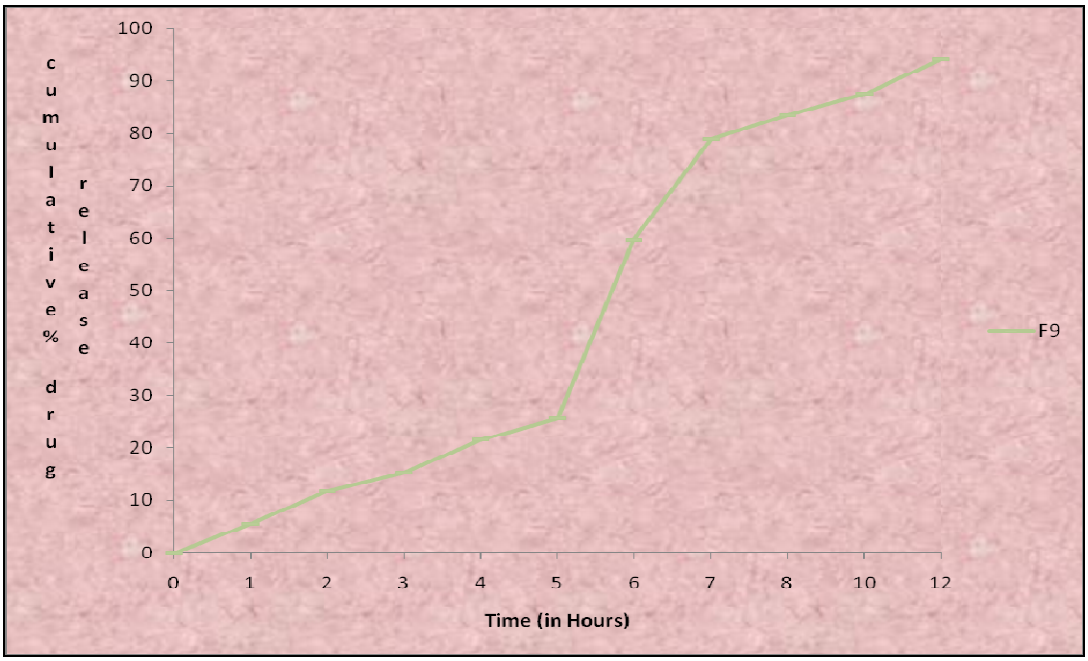


Fig.9.40. *In-vitro* drug releaseprofile of formulation F9 at the end of 2 months of stability

9.7.3. Stability studies at the end of Third month (90 days):

9.7.3.1.Drug content:

The Percentage drug content of f9 microcapsules after Third month of stability studies was studied. The results are within the official limits. The data is shown inTable 9.27.

Table 9.27: Drug content of formulation F9 at the end of 3 months of stability

Sl. No.	Formulation	Percentage drug content
1.	F9	73.89±0.036

All the values are expressed as a mean ±SD., n = 3

9.7.3.2.*In-vitro* drug release study:

The Cumulative Percentage Drug Release from F9 micro capsules after Two months of stability was studied.The data is shown in Table 9.28.

Table 9.28: *In-vitro* drug release data of formulation F9 at the end of 3 months of stability.

S. No.	Medium	Time (hours)	% Drug Release	Amount of drug released (mg)	MDT (hrs)	Cumulative % drug Release
1	0.1N HCl	0	0	0.00	0.00	0
2		1	5.62	5.62	0.50	5.62
3	pH 6.8 phosphate buffer	2	6.35±0.015	6.35	0.57	11.97
4		3	9.75±0.010	9.75	1.03	15.37
5		4	16.15±0.020	16.15	1.79	21.77
6		5	20.00±0.015	20.00	2.22	25.62
7		6	55.22±0.025	55.22	4.12	60.84
8		7	72.38±0.025	72.38	4.71	78
9		8	79.89±0.021	79.89	4.87	85.51
10		10	82.7±0.020	82.7	5.08	88.32
11		12	88.31±0.015	88.71	5.16	93.93

All the values are expressed as a mean ±SD., n = 3

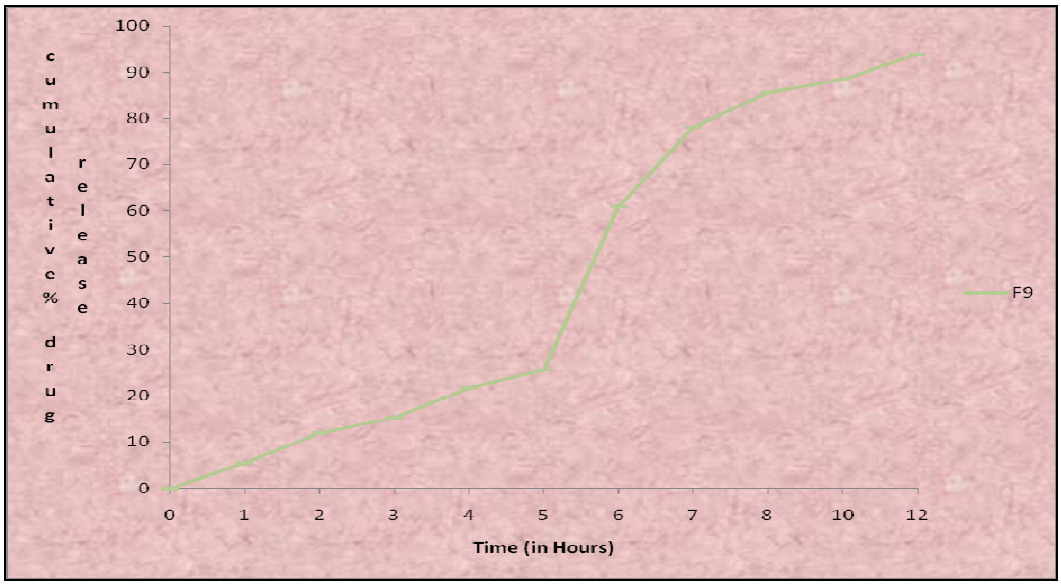


Fig.9.41. In-vitro drug releaseprofile of formulation F9 at the end of 3months of stability

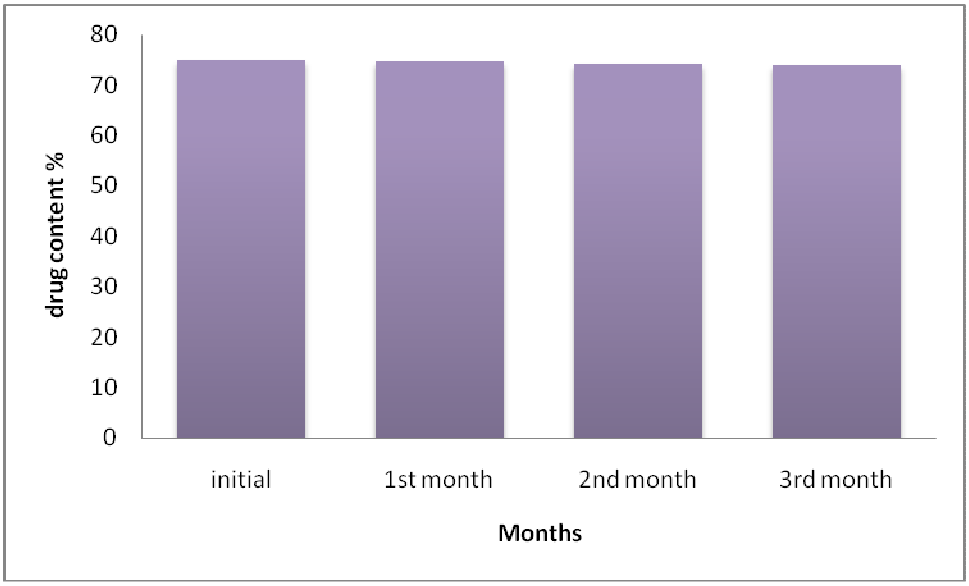


Fig.9.42. Comparisonof drugcontent for formulation F9 with initial and different periods of stability

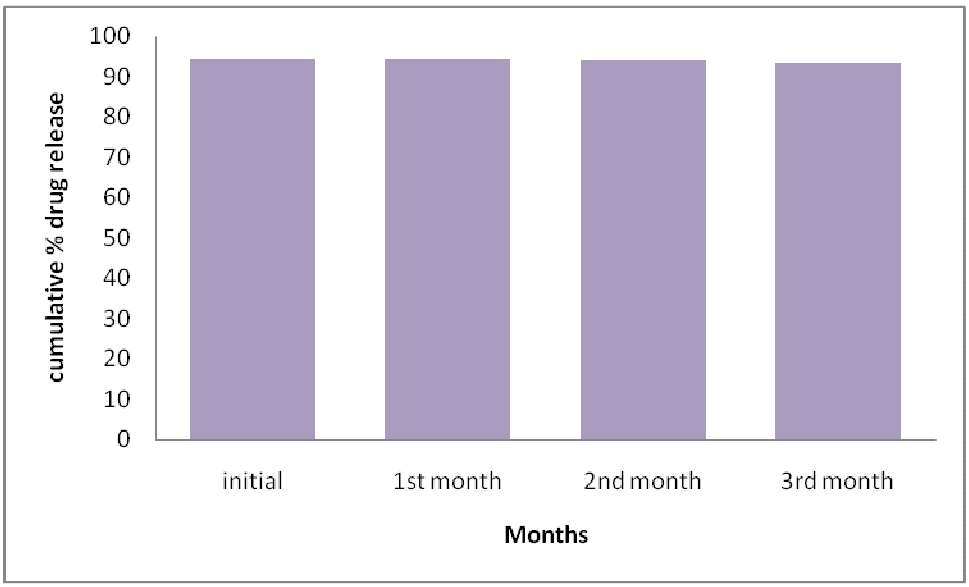


Fig.9.43. Comparison of cumulative percentage drug released at the end of 12 hours for formulation F9with initial and different periods of stability

No statistically significant differences were observed inpercentage drug content and cumulative percentage drug release in optimized formulation at the end of three months of stability studies. So it can be concluded that the formulation is stable for short term storage conditions.

SUMMARY &

CONCLUSION....

10. SUMMARY AND CONCLUSION

A successful Sustained drug delivery system was developed with the triggering mechanism that responds to the physiological conditions particular to colon.

The Sustained release microcapsules of 5-fluorouracil were prepared by using polymers like sodium alginate, gelatin and ethyl cellulosefor the treatment of colon cancer. The dissolution study of F9 Microcapsules containing Ethylcellulose was concluded the best formulation among other formulations, which showing the most desired drug release. It will be considered as optimized formulation.

The optimized formulation F9 was subjected for stability studies, the formulation was found to be stable in short term stability study.

From the *in-vitro*drug release data, it can be concluded that the Ethyl cellulose are capable of protecting the drug from being released in Stomach and in Small Intestine. This retardant capacity is more in F9 as compared to other formulations.

During the *in-vitro* drug release study, on exposure to the dissolution fluid, the microcapsules slows down further seeping-in of dissolution fluids towards the interior of the capsules. Once the gel layer is formed the drug release takes place mainly by diffusion from the inner region. On reaching the colonic environment the polymeric layer would soluble at colonic pH and release the major amount of drug in the region of colon.

In the *in-vitro* drug release study, the drug release from the microcapsules required a longer time in experimental conditions. But in actual use in living systems these limitations for pH environment and it will never be felt. Therefore the microcapsules will be take place completely and rapidly in the colon region.

While analyzing the drug release pattern of the drug from the microcapsules, it was found that the drug release started in the early hours of study. This was due to change in pH.

Out of the nine formulations, it appears that F9 has the maximum potential in providing controlled drug delivery.

FUTURE PROSPECTS....

11. FUTURE PROSPECTS

In this work only physic-chemical characterization and *in-vitro* evaluation of 5-fluorouracilmicrocapsules were done.

1. Along with in-vitro release study in-vivo release studies are also important. So in future in-vivo release study using different models are required to set the *in-vitro in-vivo* correlation which is necessary for development of successful formulation and also long term stability studies are necessary.
2. Study the effect of various geometric shapes, in a more excessive manner than previous studies, extended dimensions.
3. Design of novel polymers according to clinical and pharmaceutical need.

BIBLIOGRAPHY....

12. BIBLIOGRAPHY

1. A. V Yadav, A. S. Shete, A.P. Dabke and V.R. Shinde, Formulation and In-vitro Evaluation of Aceclofenac Microcapsules, International Journal of Pharm tech Research, **2009** April-June; vol.1, No.2, pp 135-138.
2. Adimoolam Senthil, Thakkar Hardik Kumar Rajesh Bhai, Dave Mehulkumar Vinod Bhai, Lad Tejas Bharat Bhai and Bhagya Lakshmi Battu. Formulation and Evaluation of Mucoadhesive Microspheres of Venalafaxine HCl, *International Research Journal of Pharmacy,* **2009,** 2(4), 194-199.
3. Alaa Eldeen Bakry Yassin, Ibrahim A. Alsarra. Farsk,Alanazi, Abdullah M. Al-Mohizea, Abdulrahman A.Al-Robayan and Omar A.Al-obeed, New targeted- colon delivery system: invitro and invivo evaluation using X-ray imaging , Journal of drug targeting, **2010**, 18(1), 59-66.
4. Anonymous, Indian Pharmacopeia, The Controller of Publication, Govt. of India. Ministry of Health and Family Welfare, New Delhi. Vol I, **2007**, 150, 515-516.
5. Anonymous, www.Fluorouracil - Wikipedia, the free encyclopedia.html
6. Anonymous, www.fluorouracil/DrugBank Fluorouracil (DB00544).html
7. Anonymous, www.fluorouracil/DrugBank Fluorouracil (DB00544).html
8. Asija Rajesh, Rathi Harish and Asija Sangeeta. Sustained Released Drug Technology: A Review *International Journal of Research in Pharmacy and Science,* **2012**, 2(4), 1-13.
9. Bansode S. S, Banarjee S. K, Gaikwad D. D, Jadhav S. L and Thorat R. M. Microencapsulation: A Review, *International Journal of Pharmaceutical Sciences Review and Research,* **2010**, 1(2),38-43.

10. Brahmankar DM, Jaiswal BS, Text book of Biopharmaceutics and pharmacokinetics, 1st edition. Delhi, Vallabh Prakashan, **2005**; 335-340.
11. Caugh Isha, Seth Nimrata, Rana A.C and Gupta Surbhi. Oral Sustained Release Drug Delivery System: An Overview, *International Research Journal of Pharmacy*, **2012,** 3(5), 57-62.
12. Chein YW, Novel drug Delivery system, 2nd Edn., Marcel Dekker inc, New York, US, **2009**, 1-21,115-117.
13. Christopher S. Brazel, Nikolaos A. Peppas. Modeling of drug release from swellable polymers, *European Journal of Pharmaceutics and Biopharmaceutics*, 49, **2000**, 47-58.
14. Donald LW, Hand book of Pharmaceutical controlled release tecnology, Marcel Deker Inc., Newyork, **2000**, 583-583.
15. Khachane K.N, Gaikwad P.D and Pawar S.P. Novel Sustained Release Drug Delivery System: Review, International Journal of Pharmaceutical Research and Development, **2011,** 3(12), 1-14.
16. Kranthi Kumar Kotta, Dora Babu N and Pasupathi A. Design and evaluation of multi particulate system of extended release indomethacin capsules USP, *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **2010**, 1(4), 74-82.
17. Krishnaiah YS, Satyanarayana V, Dinesh Kumar B and Karthikeyan RS, In vitro drug release studies on guar gum-based colon targeted oral drug delivery systems of 5-fluorouracil, *Europian Journal Pharmaceutical Sciences*. **2002** Aug;16(3), 185-92.

18. L. Lachman, H.A. Liberman and J.L. Kanig, Microencapsulation, The theory and practice of Industrial Pharmacy, Varghese publishing house., Bombay, **1991**, 3, 412-425.
19. Leon, L., Herbert A. L and Joseph, L. K. " The Theory And Practice Of Industrial Pharmacy", 3rd edition, **1990,** Varghese Publishing House,412, 428.
20. Nitika Agnihotri, Ravinesh Mishra, Chirag Goda and Manu Arora. Microencapsulation – A Novel Approach in Drug Delivery: A Review, *Indo Global Journal of Pharmaceutical Sciences*, **2012**, 2(1): 1-20.
21. Prakash K, Raju P N, Shanta K K, and Lakshmi M N. Preparation and characterization of lamivudine microcapsules using various cellulose polymers, *Tropical Journal of Pharmaceutical Research,* **2007***,* 6(4), 841-847.
22. Puratchikody A, Prasanth V.V, Sam T. Mathew and Ashok Kumar B. Buccal Drug Delivery: Past, Present and Future – A Review *International Journal of Drug Delivery*, (3) **2011**, 171-184.
23. Raymond C Rowe, Paul J Sheskey and Marian E Quinn, Hand book of pharmaceutical excipients, 6th edn, London, pharmaceutical press, **2009,** 262, 278, 622.
24. Sampath Kumar K. P, Debjit Bhowmik, Chiranjib, Margret Chandira and Tripathi K. K. Innovations In Sustained Drug Delivery System and Its Market Opportunities, *Journal of Chemical and Pharmaceutical Research*, **2010**, 2(1), 349-360.

25. Shaik. Shabbeer, Shaheeda and KV. Ramanamurthy, formulation and evaluation of chitosan sodium alginate microcapsules of 5-fluorouracil for colorectal cancer, ijrpc 2012, 2(1) 7-19.
26. Shalin A. Modi, Gaikwad P. D, Bankar V. H and Pawar S. P. Sustained Release Drug Delivery System : A Review**,** *International Journal of Pharma Research and Development,* **2011**, 2(12), 147-160.
27. Sonia gupta, pms bedi and neena bedi, devolapment and in vitro evaluation of eudragit rs 100 and insulin coated pectin matrix tablets of 5-fluorouracil for colon targeting, *International Journal of applied pharmaceutics*. **2010** 2(1):1-6.
28. Ujwala Shinde and Mangal Nagarsenker. Microencapsulation of euginol by gelatin-sodium alginate complex coacervation, *Indian Journal of Pharmaceutical Sciences*. **2011** May-Jun; 73(3): 311-315.
29. Vaghani SS, Jivani NP, Serasia TH, Vasanti S, Satish CS and Patel MM, preparation an characterization of 5-fu loaded microspheres of eudragit and ethyl cellulose, Arspharm, **2011**, 52(1), 23-30.